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THESIS

ANTIBACTERIAL POTENTIAL OF THAI MEDICINAL PLANTS

WUT WUTITHAMAWECH

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
Doctor of Philosophy (Argicaltural Research and Development)
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Wut Wutithamawech 2014: Antibacterial Potential of Thai Medicinal Plants. Doctor of Philosophy (Agricultural Research and Development) Major Field: Agricultural Research and Development, Faculty of Agriculture at Kamphaeng Saen. Thesis Advisor: Assistant Professor Ponsiri Liangsakul, Ph.D. 108 pages.

Ethanol and hexane extracts of twenty Thai medicinal plants were screened *in vitro* for antibacterial activitiy against *Bacillus cereus, Escherichia coli* and *Staphylococcus aureus* using disc diffusion method. The ethanol extracts of *Clerodendrum inerme* (L.) Gaertn., *Combretum qurdrangulare* Kurz, *Psidium guajava* L., and *Stephania venosa* (Blume) Spreng. showed antibacterial activity against all three pathogens tested. Based on minimum inhibitory concentration (MIC) of these four medicinal plant extracts, the ethanol extract of *C. inerme* demonstrated the highest antibacterial potency. The determination of MIC and minimum bactericidal concentration (MBC) of the extract using microdilution method showed that such extract had MIC for *B. cereus, E. coli*, and *S. aureus* of 0.039, 0.312 and 0.156 mg/ml, respectively, and MBC for *B. cereus, E.coli*, and *S. aureus* of 0.039, 0.625 and 0.312 mg/ml, respectively. Polyphenolic compounds presented in the ethanol extract of *C. inerme* roots included saponins, flavonoids, and alkaloids. The ethanol crude extract of *C. inerme* roots was subsequently used in developing a *C. inerme* antibacterial gel. Carbopols Ultrez 10 was used as a gel base. The crude extract was added into the gel base at different concentrations. Determination of MBC of the gel product at 3- and 30- min exposure time against *B. cereus, E. coli* and *S. aureus* was carried out. It was found that the treatment of 5 mg *C. inerme* extract/100g gel for 30 min was effective as it showed killing activity on all three pathogens; it killed 99.8% *B. cereus*, 94% *E. coli* and 99.2% *S. aureus*. This study also confirmed the potential of Thai medicinal plants as ingredients for medicines to be used in the Thai traditional medical practice.

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LIST OF ABBREVIATIONS

°C	degree celcius
CFU/ml	colony forming unit/milliliter
cm	centimeter
DMSO	dimethylsulfoxide
g	gram
h	hour
l	liter
MBC	minimum bactericidal concentration
MIC	minimum inhibitory concentration
min	minute
µg	microgram
µl	microliter
ml	milliliter
mM	milli molar
mg	milligram
MRSA	methicillin-resistant *Staphylococcus aureus*
NaCl	sodium chloride
ppm	part per million
rpm	round per minute
RT	room temperature
v/v	volume/volume
w/v	weight/volume
w/w	weight/weight

ANTIBACTERIAL POTENTIAL OF THAI MEDICINAL PLANTS

INTRODUCTION

Infectious diseases are considered to be one of the major health problems in Thailand as well as other developing countries (Ahmad and Arina, 2000; Trakulsomboon *et al.*, 2006). The treatment of these infections is mainly based on the uses of antibiotics (Kowti *et al*., 2010). Antibiotic resistance has become a global concern (Parekh *et al*., 2005). Multiple drug resistant bacteria have developed due to the indiscriminate uses of commercial antimicrobial drugs (Gislene *et al.*, 2000). It is the major cause of failure in the treatment of infectious diseases (Davis, 1994). The increasing failure of antibiotics has led to the screening of medicinal plants for their potential antimicrobial activity (Parekh and Chanda, 2007).

According to the World Health Organization, medicinal plants would be the best source to obtain a variety of drugs (Gislene *et al.*, 2000). The advent of science into the search for antibiotics largely depends on some of these plants as raw materials (Jayalakshmi *et al.*, 2011). Thailand is endowed with a rich biodiversity of plant species and it has a long history of herbal medicine practices (Wiyakrutta *et al*., 2004). Plenty of Thai medicinal plants are still commonly used in a wide range of clinical settings for the treatment of infectious diseases (Trakulsomboon *et al*., 2006).

Three microorganisms, namely *Bacillus cereus*, *Escherichia coli*, and *Staphylococcus aureus*, are commonly used for preliminary investigation of possible antibacterial activity of plant extracts.

Bacillus cereus, a Gram-positive spore-forming rod-shaped bacterium, causes two different forms of food poisoning, an emetic illness and a diarrhoeal illness. It is also associated with septicemia, respiratory infection, pneumonia, meningitis, wound and ocular infection (Olawale and Akintobi, 2011). It is regarded as one of the major microbiological hazards in the food industry (Schlegelova *et al*., 2003). It has also

been reported to develop overall resistance to several antibiotics (Whong and Kwaga, 2007).

Escherichia coli is a Gram-negative, rod-shaped bacterium found in the lower gastrointestinal tract. It may cause urinary, wound, lung, meningeal and septicaemic infections (Spicer, 2008). The urinary tract infection is complicated by the increasing incidence of infections caused by various strains of *E. coli* resistant to commonly used antimicrobial agents (Manges *et al*., 2001). *E. coli* is sometimes used as a sentinel for monitoring antimicrobial drug resistance in fecal bacteria because it is found more frequently in a wide range of hosts, acquires resistance easily, and is a reliable indicator of resistance in salmonellae (Tadesse *et al*., 2012).

Staphylococcus aureus is a Gram-positive, spherical shape bacterium, one of the major human pathogens, causing abscesses in skin and most other organs leading to bacteraemia and endocarditis and also produces many toxins (Spicer, 2008). Incidence of *S. aureus* infection has increased during the past two decades. Bacteremia due to *S. aureus* has been reported to be associated with mortality rates of 15–60% (Cosgrove *et al*., 2003). *S. aureus* is among the most prevalent causes of clinical infections globally and has garnered substantial public attention due to increasing mortality associated with multi-drug resistance (Waters *et al.*, 2011).

In this study, 20 medicinal plants (belonging to 16 families) traditionally prescribed in Thailand for the treatment of infectious diseases were selected for antibaterial screening. The activity was assayed by disc diffusion method, together with antibacterial susceptibility test. Inhibitory activity of the plant extract which showed the highest antibacterial activity against all tested bacteria was selected for further studies, including determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

According to the Thai traditional medicine practice, drugs from medicinal plants were prepared in alcoholic macerate form (Saralamp *et al.* 1996). In this study,

ethanol and hexane were used as extraction solvents to investigate the efficacies of Thai medicinal plants.

OBJECTIVES

1. To investigate antibacterial activities of twenty medicinal plants

2. To develop an antibacterial product from potential Thai medicinal plants

LITERATURE REVIEW

1. Thai Medicinal Plants

Numerous Thai medicinal plants are still commonly used in a wide range of clinical settings to treat infectious diseases. They have long been used and prescribed in Thailand for centuries, such as:

1.1 *Albizia chinensis* (Osbeck) Merr.

Family: MIMOSACEAE
Voucher number: BKF: 154214
Common name: Chinese albizia, silktree, tamaligi
Thai name: Kang luang (คางหลวง)
Part used: roots

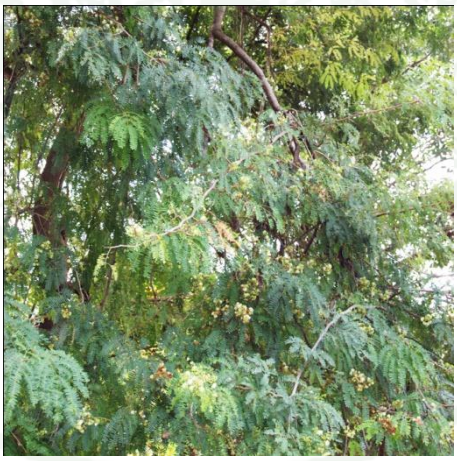

Figure 1 *Albizia chinensis* (Osbeck) Merr.

Description: A large deciduous tree with tall cylindrical bole and spreading crown. Leaves: abruptly 2-pinnate; pinnae: 15-25; leaflets: 40-80; stipules and bracts: persistent. Flowers: small, yellowish, in small panicled heads, pale brown (Smitinand and Larsen, 1985).

Traditional uses: Use in the treatment of infected wound and abscess (Pongboonrod, 2493). Applied in the treatment of diarrhea, hemorrhoid, leukorrhea with infection, abnormal urine, hematuria and hemoptysis (Veeraphajuk *et al.*, 2545).

Chemical constituents: Eight compounds were isolated from the ethanol extract of the leaves of *A. chinensis* and their structures were elucidated as quercetin 3'-O-β-D-glucopyranosyl-3-O-rutinoside, kaempferol 3, 7-di-O-β-D-glucopyranoside, rutin, D-pinitol, luteolin 7-O-β-D-glucopyranoside (5), (+)-lyoniresinol 3α-O-β-D-glucopyranoside, (-)-lyoniresinol 3α-O-β-D-glucopyranoside, syringin (Liu *et al*., 2009b). Three new oleanane-type triterpene saponins, albizosides A-C (1-3), were isolated from the stem bark of *A. chinensis*. Their structures were established by 1D and 2D NMR experiments and chemical methods. Compounds 1-3 showed cytotoxic activity against a small panel of human tumor cell lines as well as hemolytic activity against rabbit erythrocytes (Liu *et al*., 2009a).

1.2 *Catunaregam tomentosa* (Blume ex DC.) Tirveng.

Family: RUBIACEAE

Voucher number: BKF: 154226

Common name: Lemon-scented Darwinia

Thai name: Ma khet (มะเค็ด)

Part used: roots

Figure 2 *Catunaregam tomentosa* (Blume ex DC.) Tirveng.

Description: Small tree, 4-8 m high; branches: long and thorny. Leaves: simple, opposite, broadly ovate or ovate-elliptic, 3-5 cm wide, 5-7 cm long, pale coloured and pubescent beneath; stipules: interpetiolar. Flowers: solitary, axillary; corolla whitish, fragrant. Fruits: dry, dehiscent, ovate (Chuakul *et al*., 1997).

Traditional uses: Applied in the treatment of granular stomatitis, aphthous ulcer, cancerous or non-cancerous chronic ulcers (Veesapen *et al*., 2548a).

Chemical constituents: Five hybrid peptide-polyketides, curvularides A-E (1-5), were isolated from the endophytic fungus *Curvularia geniculata*, which was obtained from the limbs of *C. tomentosa*. Structure elucidation for curvularides A-E (1-5) was accomplished by analysis of spectroscopic data, as well as by single-crystal

X-ray crystallography. Curvularide B (2) exhibited antifungal activity against *Candida albicans*, and it also showed synergistic activity with a fluconazole drug (Chomcheon *et al*., 2010).

1.3 *Cleome viscosa* L.

Family: CAPPARIDACEAE
Voucher number: BKF: 154215
Common names: Stinking cleome, Wild caia, Tickweed, Wild mustard
Thai name: Phak sian phi (ผักเสี้ยนผี)
Part used: whole plant

Figure 3 *Cleome viscosa* L.

Description: Annual herb, up to 1 m high; densely glandular pubescent and viscid throughout. Leaves: palmately compound, alternate; leaflets: 3-5, obovate or ovate, 1-1.5 cm wide, and 1.5-4.5 cm long. Inflorescence: in terminal raceme. Flowers: yellow. Fruits: silique, dehiscing from the base. Common as a weed of disturbed places, near sea level, in cultivated fields, along roadsides, and especially in canefields, mostly a roadside plant (Chuakul *et al*., 1997).

Traditional uses: Applied in the treatment of tuberculosis of spine, inflammation of Pattakaat line (S.S., 2483), abscess (Traditional medical school faculty, 2505; Veesapen, 2548b). Antiflatulent, antidarrheal, antipyretic, fire element tonic, treatment of abdominal pain, abscess or infected inflammation, tumor and cancer in lung, intestine or liver. Flowers: antiseptic. Fruits: anthelmintic. Roots: treatment of uterine infection for post-labor (Chuakul *et al*., 1997).

Chemical constituents: Terpenoids were earlier reported to be present in species of *Cleome*. Terpenoids and glucosinolates such as glucocapparin and glucocleomin have been reported to be the major organoleptic compounds in *C. viscosa* (Songsak and Lockwood, 2002). Quercetin 3-*O*-(2″-acetyl)-glucoside obtained from ethyl acetate fraction of *C. viscosa* showed significant anti-inflammatory activity on carrageenan-induced rat paw edema (*in vivo*) and anti-microbial activity (*in vitro*) on *S. aureus* and *E. coli*. Selective toxicity with flavonoid glycoside towards the Gram-positive bacteria was found on *S. aureus*. The structure of the glycoside confirmed by means of hydrogen-1 nuclear magnetic resonance spectroscopy, carbon nuclear magnetic resonance spectroscopy, attached proton test, and mass spectrum (Senthamilselvi *et al.*, 2012).

1.4 *Clerodendrum inerme* (L.) Gaertn.

Family: VERBENACEAE

Voucher number: BKF: 154225

Common names: Seaside clerodendron, Garden quinine, Wild jasmine

Thai name: Samma nga (สำมะงา)

Part used: roots

Figure 4 *Clerodendrum inerme* (L.) Gaertn.

Description: An erect or somewhat struggling shrub of 1 to 4 m high. Leaves: ovate, oblong-ovate or elliptic ovate, 4 to 8 cm long, 2 to 5 cm wide, shining, smooth, entire and pointed at the tip. Inflorescence (cyme): usually composed of three flowers, borne in the axils of the leaves. Calyx: green, narrowly funnel-shaped, and furnished with 5 very short teeth. Corolla: about 3 cm long and comprises a slender, white tube spreading, purple-tinged lobes about 7 mm long. Stamens: long-exserted, and purple. Fruits: obovoid, about 1.5 cm long, and splitting into 4 pyrenes. Calyx in the fruit is about 1 cm in diameter.

Traditional uses: Use in the treatment of skin diseases with pruritus (Pongboonrod, 2493), gout, hepatitis, wound inflammation and swelling,

rheumarthritis, atopic dermatitis and cold (Boonyaphapas and Chokchaipon, 2543).

Chemical constituents: Phytochemical studies revealed the presence of flavanoids, sterols, flavones, triterpenes, diterpenes, quinone and neolignans. Study of hexane extract of the aerial parts isolated an aliphatic glucoside characterized as pentadecanoic acid-ß-D-glucoside. A butanol extract yielded acacetin and apigenin. Aerial parts yielded a new clerodane diterpenoid, cleroindermin, and a known flavonoid, apigenin (Boonyaphapas and Chokchaipon, 2543).

Studies: Methanol and benzene extracts of *C. inerme* (leaves and roots) exhibited varying degrees of inhibitory effect against tested pathogenic strains *in vitro*. None of the activity was observed in aqueous extracts. The most susceptible bacterium and fungi were *S. aureus* and *A. niger* respectively. The MIC of crude extracts of leaves and roots was determined at the concentrations ranging from 0.078 to 0.625 mg/ml. Crude methanol extract of *C. inerme* (leaves and roots) showed more pronounced antimicrobial activity than other extracts. The methanol leaves extract exhibited highest zone of inhibition against *S. aureus* and *A. niger* with low MIC values (0.78 mg/ml for each). The methanol root extract was also found to effective against *S. aureus* and *A. niger* with low MIC values (0.78 mg/ml and 0.156 mg/ml, respectively) (Chahal *et al*., 2010).

1.5 *Combretum qurdrangulare* Kurz

Family name: COMBRETACEAE

Voucher number: BKF: 154216

Common names: Takeo bushwillow

Thai name: Sakae (สะแก)

Part used: roots

Figure 5 *Combretum qurdrangulare* Kurz

Description: Tree: 5-l0 m high; young briinchlets acutely quadrangular or very narrowly quadrialate. Leaves: simple, opposite, elliptic or obovate 3-8 cm wide, 6-15 cm long; petiole: acutely ridged. Inflorescence: in terminal and axillary spike. F1owers: small, yellowish white. Fruits: dry, thinly quadrialate. Seeds: brownish red, ellipsoid, 4-angled (Saralamp *et al*., 1996).

Traditional uses: Use in the treatment of venereal diseases, anthelmintic (Saralamp *et al*., 1996); gonococcal and syphilitic arthritis, bubo and vinereal ulcers (Pongboonrod, 2493). Applied in the treatment of carcinoma of oesophagus, diarrhea. (Traditional medical school faculty, 2505). Seeds are used as anthelmintic for roundworm, threadworm in children. Preparation: grind 3 g of seeds, mix with an egg,

fry, and take once when the stomach is empty. Roots are used in the treatment of venereal diseases; anthelrnintic. Leaves relieve muscular pain (Saralamp *et al*., 1996).

Chemical constituents: Methanol extract of *C. quadrangulare* leaves contained fifteen new cycloartane-type triterpenes, methyl quadrangularates A-D and N-P, methyl 24-epiquadrangularate C, quadrangularic acid E, 23-deoxojessic acid, 1-*O*-acetyl-23-deoxojessic acid, quadragularols A and B and norquadrangularic acid B and C, together with two known cycloartane-type triterpenes, methyl 23-deoxojessate and 4β,14α-dimethyl-5α-ergosta-9β, 19-cyclo-24(31)-en-3β-hydroxy-4α-carboxylic acid. Betulinic acid, β-sitosterol, kamatakenin, isokaempferide, 5,7,4'-trihydroxy-3,3'-dimethoxyflavone and 5,4'-dihydroxy-3,7,3'-trimethoxyflavone were also obtained from the same extract. All the isolated compounds were tested for their cytotoxicity towards highly liver metastatic murine colon 26-L5 carcinoma cells. The cycloartane-type triterpenes showed various degrees of cytotoxicity, whereas all the flavonoids possessed strong cytotoxicity with ED_{50} values equaled to or less than 6 μM. Nine new cycloartane triterpenes, combretanones A-G, combretic acidA and B, were isolated from a methanol extract of *C. quadrangulare* leaves (Toume *et al*., 2011).

1.6 *Dioecrescis erythroclada* (Kurz) Tiveng.

Family name: RUBIACEAE

Voucher number: BKF: 154234

Common Names: Ma khang daeng

Thai name: Ma khang daeng (มะคังแดง)

Part used: roots

Figure 6 *Dioecrescis erythroclada* (Kurz) Tiveng.

Description: Trees: 6-12 m high; stems and branches: reddish brown; stems: aculeate. Leaves: simple, opposite, elliptic or obovate, 8-15 cm wide, 15-22 cm long; stipules: interpetiolar. Inflorescence: in upper axillary cyme; flowers: greenish yellow. Fruits: fleshy, elongate ellipsoid, with persistent calyx (Chuakul *et al*., 1997).

Traditional uses: Use in the treatment of cancerous or non-cancerous chronic ulcers, yaws, leprosy and eczema (Pongboonrod, 2493). Applied in the treatment of weeping scabiasis, abscess (Traditional medical school faculty, 2505; Veesapen, 2548b)

Chemical constituents: Six compounds were isolated from the leaves and branches of *D. erythroclada* and identified as apodanthoside, mussaenoside, gardenoside, benzyl alcohol O-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside,

phenethyl alcohol O-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside, and oct-1-en-3-ol α-l-arabinopyranosyl-(1→6)-β-D-glucopyranoside. The structures were determined based on physical data and spectroscopic evidence (Kaewkrud *et al*., 2007).

1.7 *Diospyros mollis* Griff.

Family name: EBENACEAE
Voucher number: BKF: 154210
Common Names: Ebony Tree
Thai name: Ma kluea (มะเกลือ)
Part used: roots

Figure 7 *Diospyros mollis* Griff.

Description; Trees: up to 30 m high. Leaves: simple, alternate, ovate or ovate-elliptic, 2-3 cm wide, 4-7 cm long, dark green. Flowers: solitary in pistillate flower and about 3-flowered cymose fascicle in staminate inflorescence; flowers are yellow. Fruits: berry, subglobose, 4-persistant sepals, green when young, black after ripening; seeds are few (Saralamp *et al*., 1996).

Traditional uses: Applied in the treatment of leukorrhea, abscess, yaws, leprosy, syphilis tertiary stage, pulmonary tuberculosis, manifestation of infection

(Traditional medical school faculty, 2505). Fresh unripe fruit is used as anthelmintic for hookworm. Dose: 10 fruits for 10 years old child, increase the number of fruits equal to age of patient, maximum 25 fruits. Preparation: press in mortar, use only the juice, mix with coconut milk in a ratio of 2 teaspoonfuls of milk to 1 fruit; must be freshly prepared; take once a day, 3 hours before breakfast; if the patient does not evacuate within 3 hours, take a laxative to dispel the worm and the rest of the medicine (Saralamp *et al*., 1996).

Chemical constituents: Dihydro: dihydroxy-rhamnoside acenaphthene, α-amyrin, botulin, betulin acetate, betulinic acid, dihydroxy-dimethyl: diospyrol binaphthoquinone, diospyrol-8,8'-di-O(6-β-D-apiofuranosyl-β-D-glucopyrnoside, diospyrol, diospyrol-8,8'-di-O-β-D-glucoside, diospyros dimer 3, diospyros naphthalene 8, elliptinone, n-hentriacontane, lupenone, lupeol, mamegakinone, 2-naphthaldehyde 4,5,8-trimethoxy: naphthalene, 3-formyl-1,5,8-trimethoxy: naphthalene, 3-formyl-1-hydroxy-5,8-dimethoxy: naphthalene-1,8-diol, 3-methyl: n-nonacosane, oleanolic acid, oleic acid methyl ester, palmitic acid methyl ester, β-sitosterol, stearic acid methyl ester, taraxerol (Boonyaphapas and Chokchaipon, 2542).

1.8 *Ixora javanica* (Blume) DC.

Family name: RUBIACEAE

Voucher number: BKF: 154213

Common Names: Jungle Geranium, Glossy Ixora

Thai name: Khem thong (เข็มทอง)

Part used: roots

Figure 8 *Ixora javanica* (Blume) DC.

Description; A shrub, 3-5 m tall. Leaves: elliptical, oblong or oblong-ovate, 7.5-17 cm x 2.5-7 cm, herbaceous, base acute, apex acuminate, with 9-10 secondary veins, petiole 3-6 mm long, stipules long-awned. Flowers: inflorescence loose, short-haired, peduncle 1-4 cm long; flowers with calyx tube 0.2 mm long, lobes ovate, 0.4 mm long, corolla tube 2.5-3.5 cm long, lobes ovate, obtuse or rounded, 6-8 mm long, orange-red sometimes pink or yellow, not fragrant, anthers pale orange, style 5 mm long slightly exserted. Fruits: 10-mm diameter, reddish-purple, berries (de Padua *et al.*, 1999).

Traditional uses: *Ixora* is traditionally found to be effective against many ailments like hepatic disorder, cancer, microbial infection, pain, inflammation etc, and has been documented for various medicinal properties. Several *Ixora* species are used

in traditional medicine, e.g. as an astringent and to treat dysentery and tuberculosis. The use in China and India is widespread. An infusion of the leaves or flowers of several species is administered to treat fever, headache and colic. A decoction of the roots is used as a sedative; the roots are believed to be more potent. The internal application is based on stomachic and antiseptic properties, while external applications are based on astringent and antiseptic properties. They are usd in the treatment of mucous bloody stool or sputum, eyediseases, anasarca and fire element tonic (Pongboonrod, 2493). They are also applied in the treatment of disseminated tuberculosis (Traditional medical school faculty, 2505).

Chemical constituents: The genus *Ixora* has been reported to posses different classes of compounds mainly aromatic acrid oils, tannins, saponins, carbohydratse, fatty acids, flavanoids such as β-sitosterol, and kaempferol (Kharat *et al*., 2013).

1.9 *Lepisanthes senegalensis* (Poir.) Leenh

Family name: SAPINDACEAE

Voucher number: BKF: 154229

Common Names: Senegal cherry

Thai name: Chamma liang pa (ชำมะเลียงป่า)

Part used: roots

Figure 9 *Lepisanthes senegalensis* (Poir.) Leenh

Description: Erect shrub, 30-150 cm high. Leaves: bifoliolate, alternate; leaflets: oblong, lanceolate or narrowly oblanceolate, 1.5-3 cm wide, 8-12 cm long. Inflorescence: in terminal panicle; flowers are purplish red. Fruits: berry, subglobose, turned dark puple when ripe (Saralamp *et al*., 1997).

Traditional uses: Roots are used in the treatment of cerebral malaria, fever with vertigo, chest pain and nosebleed (Chuakul, 1997). Decoction is used for the treatment of cerebral malaria, fever with vertigo, chest pain and nosebleed. It is also used a muscular spasm relief (Saralamp *et al*., 1997).

Chemical constituents: -

1.10 *Luvunga scandens* (Roxb.) Buch.-Ham.

Family name: RUBIACEAE

Voucher number: BKF: 154228

Common Names: Lavang Lata, Indian Lavanga

Thai name: Chang nga diao (ช้างงาเดียว)

Part used: roots

Figure 10 *Luvunga scandens* (Roxb.) Buch.-Ham.

Description; Heavy woody climbers. Leaves: digitately 3-foliolate; petiole: 2-9 cm; petiolules: 3-10 mm; leaflets: blades elliptic to obovate, 6-20 × 3-9 cm. Flowers: ellipsoid in bud. Calyx: 2.5-4 mm, shallowly 4-lobed. Petals: 4.6-10 mm. Stamens: 8 or fewer. Fruits: yellow, globose or obpyriform, 3-5 cm in diameter, smooth surface, 1-4-seeded; thick outer part of pericarp (exocarp and mesocarp). Seeds: broadly ovoid, 2-3 cm. (Zhengyi *et al*., 2013).

Traditional uses: Use in the treatment of internal and external abscess, disorders of urination and neohropathy (Pongboonrod, 2493).

Chemical constituents: -

1.11 *Pavetta indica* L.

Family name: RUBIACEAE

Voucher number: BKF: 154219

Common Names: Indian Pavetta, Pavetta

Thai name: Khem pa (เข็มป่า)

Part used: roots

Figure 11 *Pavetta indica* L.

Description; Shrubs, 1-3 m tall; young branches: compressed to subterete, glabrescent or puberulent. Petiole: 10-30 mm, puberulent; leaf blade: drying membranous, narrowly obovate or lanceolate, 9-13×3.5-4.7 cm, with several bacterial nodules, adaxially glabrescent, abaxially puberulent at least along veins, base cuneate or acute, apex acuminate; secondary veins: 6-8 pairs; stipules: ovate-triangular, 5-7 mm, puberulent or glabrescent, shortly aristate. Inflorescences terminal on developed branches, laxly corymbose, ca. 9×15 cm, many flowered, strigillose to glabrescent; peduncles: 1.5-2 cm; pedicels 3-5 mm. Flowers: pedicellate. Calyx: with hypanthium portion ellipsoid, 1-1.2 mm, densely strigillose; limb: 1-1.5 mm, sparsely strigillose, lobed for up to 1/2. Corolla: white, outside glabrous; tube: 19-22 mm, bearded in throat; lobes: narrowly ligulate, 6-7 mm, obtuse to round. Style: ca. 30 mm. Drupe: globose, ca. 8 mm, glabrous (Barkle, 2006).

Traditional uses: Use in the treatment of bronchial sputum occlusion, mucus in the gastrointestinal tract and blepharitis (Pongboonrod, 2493). Applied in the treatment of gastroenteritis (Veesapen, 2548b). The leaves and roots are employed in the preparation of poultices for boils and itches; decoctions of leaves are used as a lotion for ulcerated nose and for heamorrhoids. Roots are used for anticephalagic. Leaves are used in haemorrhoidal pain and ulcerated nose. Wood is used as antirheumatic. Fruits are used as anthelmintic (Ramamoorthy *et al*., 2010).

Chemical constituents: The chemical composition of the volatile oil (0.25%) from leaves of *Pavetta indica* Linn. indicated presence of 24 compounds. The major constituents of the oil were β-pinene (25.45%), β-eudesmol (7.06%) and tricyclene (5.74%). The oxygenated monoterpenes and sesquiterpene hydrocarbons were found in the oil as minor components. The yield of essential oil obtained from aerial parts were 0.05% (v/w) (Prasad et al., 2011).

1.12 *Phyllanthus emblica* L.

Family name: EUPHORBIACEAE

Voucher number: BKF: 154222

Common Names: Indian gooseberry, Amlaki

Thai name: Makham pom (มะขามป้อม)

Part used: roots

Figure 12 *Phyllanthus emblica* L.

Description; Trees: 8 to 20 m high. Leaves: simple, alternate, oblong, 0.25-0.5 cm wide, 0.8-1.2 cm long. Inflorescence: in fascicle with staminate cluster at base and pistillate flower upwards, axillary; flowers: white. Fruits: drupe, globose, glabrous, with six longitudinal lines; endocarp: globose, pale green; seeds: 6 (Saralamp *et al*., 1996).

Traditional uses: Applied in the treatment of carcinoma of base of tongue (S.S., 2483), splenomegaly caused by malaria (Veesapen, 2548a), urethral polyps, intestinal parasites and cancerous or non cancerous chronic ulcers (Veesapen, 2548b); felon (Veesapen, 2548c).

Chemical constituents: ascorbic acid, astragalin, chebulagic acid, chibulinic acid, corilagin, ellagic acid, emblicol, gallic acid, gallic acidethyl ester, digallic acid, glucogallin, 1,6-di-O-galloyl-β-D-glucose, gibberellin A-1, gibberellin A-3, gibberellin A-4, gibberellin A-7, gibberellin A-9, 3,6-di-O-galloyl glucose, myo-inositol, kaempferol, leucodelphindin, linoleic acid, linolenic acid, lupeol, myristic acid, oleic acid, palmitic acid, phyllantidine, phyllantine, phyllemblic acid, polysaccharide, putranjivain A, qeurcetin-3-O-β-D-glucoside, quercetin; rutin, β-sitosterol, stearic acid, tannin, zeatin, zeatin nucleotide, zeatin riboside (Boonyaphapas and Chokchaipon, 2542).

1.13 *Polyalthia cerasoides* (Roxb.) Benth. ex Bedd.

Family name: **ANNONACEAE**
Voucher number: BKF: 154232
Common Names: Kudumi
Thai names: Phaya rak dam (พญารากดำ)
Part used: roots

Figure 13 *Polyalthia cerasoides* (Roxb.) Benth. ex Bedd.

Description; Medium-sized tree, 12-20 m high. Leaves: simple, alternate, lanceolate or elliptic, 2.5-5 cm wide, 6.5-15 cm long. Flowers: solitary or 2-3

flowered fascicled; corolla: yellowish green, slightly pubescent. Fruits: aggreegate, ovoid, turned red whenripe (Chuakul *et al*., 1997).

Traditional uses: Use in the treatment of muscular pain (Chuakul, 1997), abscess, tuberculosis (Pongboonrod, 2493). Applied in the treatment of severe diarrhea (Veesapen, 2548b), massive hepatomegaly, single apex abscess (Traditional medical school faculty, 2505). Roots are antipyretic. Decoction of the roots is used for anemia, along with pale, muscular pain and weight loss. It is also used as a contraceptive for women and a tonic for men (Chuakul *et al*., 1997).

Chemical constituents: Chromatographic separation of the crude methanol extract of roots led to the isolation of four isoquinoline alkaloids; laudanosine, codamine, laudanidine and reticuline. The structures of these compounds were identified by spectroscopic methods. Bioactivity assays revealed that compounds 2 and 3 showed activities against malaria with IC_{50} values of 4.24 and 7.02 μg/mL, respectively (Lekphrom *et al*., 2008). Stem bark methanol extract was previously documented. The isolation of bioactive N-(4-hydroxy-β-phenethyl)-4-hydroxycinnamide from ethyl acetate extract of the plant species included stigmasterol. A mixture of triterpenes from hexane and dichloromethane extracts was also reported. Trace essential elements were found in the hexane extract in ppm levels. The plant extracts were evaluated for their antimicrobial and antioxidative activities. The dichloromethane extract displayed the highest activity against *Corynebacterium diphtheriae* NCTC 10356 with MIC of 32 μg/mL, as well as, the highest SOD activity with an IC_{50} of 4.51 μg/mL (Treeratanapiboon *et al*., 2011).

1.14 *Pouzolzia hirta* (Blume) Hassk.

Family name: URTICACEAE

Voucher number: BKF: 154220

Common Names: Nettles

Thai names: Khop cha nang (ขอบชะนาง)

Part used: whole plant

Figure 14 *Pouzolzia hirta* (Blume) Hassk.

Description; Creeping herb, 20-30 cm high. Leaves: simple, opposite, ovate in white variety, 1-1.5 cm wide, 2-3 cm long, light green, lanceolate in red variety, 1.5-2.5 cm wide, 5-8 cm long, brownish green above, reddish purple beneath. Inflorescence: in axillary cluster, unisexual, monoecious; flowers: yellowish white in white variety, red in red variety. Fruits: achene (Saralamp *et al*., 1996).

Traditional uses: Use in the treatment of venereal diseases and worm (Pongboonrod, 2493). Apply locally for abscess as an anti-inflammatory agent (Saralamp *et al*., 1996). Applied in the treatment of disseminated tuberculosis (Traditional medical school faculty, 2505; S.S., 2483); infectious diseases in early childhood (Veeraphajuk, 2545). Decoction of the whole plant is emmenagogue,

diuretic, and insecticidal. Ground fresh leaves are applied locally for abscess as an anti-inflammatory (Saralamp *et al*., 1996).

Chemical constituents: -

1.15 *Psidium guajava* L.

Family name: MYRTACEAE
Voucher number: BKF: 154223
Common names: Guava, Lemon guava
Thai name: Farang (ฝรั่ง)
Part used: leaves

Figure 15 *Psidium guajava* L.

Description: It is a low evergreen tree or shrub 6 to 25 feet high, with wide-spreading branches and square, downy twigs. It is a native plant of tropical America. Guava is a tropical and semitropical plant. The branches are crooked, bringing opposite leaves. The flowers are white, incurved petals, 2 or 3 in the leaf axils; they are fragrant, with four to six petals and yellow anthers. The fruits are small, 3 to 6 cm long, pear-shaped, reddish-yellow when ripe (Hernandez, 1980).

Traditional uses: Used as antidiarrheal and antidysenteric (Pongboonrod, 2493; Saralamp *et al*., 1996). Leaves are antidiarrheal, antidysenteric. They are externally used as deodorant in mouth wash. Roots are diuretic and are used to treat abnormal urination. Clinical study on antidiarrheal effect showed that 500 mg dried powder taken every 3 hours for three days was better than tetracycline (Saralamp *et al*., 1996). The medicinal uses of the guava are many and varied. Stem, bark and root-bark are astringent. Unripe fruit is indigestible, causing vomiting and feverish. Bark is astringent, febrifuge, antiseptic. Fruits are laxative; leaves are astringent (Hernandez, 1980).

Chemical constituents: The leaves contain essential oil with the main components being α-pinene, β-pinene, limonene, menthol, terpenyl acetate, isopropyl alcohol, longicyclene, caryophyllene, β-bisabolene, caryophyllene oxide, β-copanene, farnesene, humulene, selinene, cardinene and curcumene [Zakaria]. Nerolidiol, β-sitosterol, ursolic, crategolic, and guayavolic acids have also been identified from the essential oil of the leaves (Iwu, 1993). Four antibacterial flavonoids (morin-3-*O*-lyxoside, morin-3-*O*-arabinoside, quercetin, and quercetin-3-*O*arabinoside) were isolated from fresh and dried leaves. They had bacteriostatic mode of action against tested spoilage and foodborne pathogenic bacteria including *B. stearothermophilus, B. thermosphacta, E. coli, L. monocytogenes, P. fluorescens, S. enterica, S. aureus* and *V. cholerae* (Rattanachaikunsopon and Phumkhachorn, 2010). The use of guava leaf water extract as antidiarrheal agent for a short period of time was suggested to be safe (Thaina *et al*. 2540).

1.16 *Scoparia dulcis* L.

Family name: SCROPHULARIACEAE

Voucher number: BKF: 154209

Common name: sweet-broom, goatweed, scoparia-weed

Thai names: Krot nam (กรดน้ำ)

Part used: whole plant

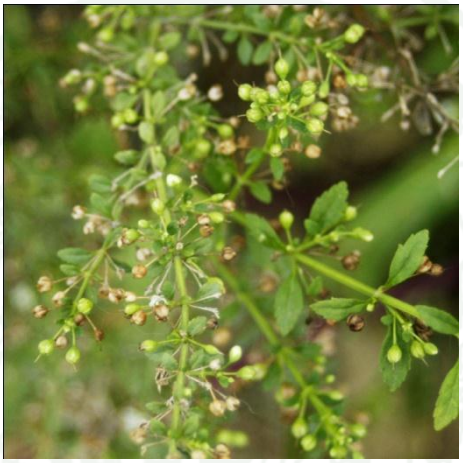

Figure 16 *Scoparia dulcis* L.

Description: The plant is an erect, shrubby herb that grows up to about 50 cm high. It usually has many auxiliary shoots and reproduces from seeds. The stem is more or less woody, ribbed, mainly branched and glabrous. The leaves are opposite or three at a node, oval or narrowly oblanceolate; about 2.5 cm to 5.0 cm long and 1.5 cm wide, widely toothed at the upper part of the leaf and wedge–shaped at the lower part of the leaf. The leaf blade is smooth except that the lower surface has some glandular dots. The inflorescence is a slender raceme with one or two flowers in the upper leaf axils. The fruit is a round capsule (Okhale *et al*. 2010).

Traditional uses: Applied in the treatment of infected wound, dysentery severe stage and diarrhea (Traditional medical school faculty, 2505). The Brazilian folkloric use is to treat bronchitis, gastric disorders, hemorrhoids, insect bites and skin wounds.

Asian medicine uses the herb to treat hypertension. The herb also has antiulcer properties. In tropical and subtropical regions, the fresh or dried plant of *S. dulcis* has traditionally been used as one of remedies for stomach troubles, hypertension, diabetes, bronchitis, and as an analgesic and antipyretic. In Nigeria *S. dulcis* is used for the management of diabetes, as love charm, and chewed to secure favor from people in authorities (Okhale *et al*. 2010).

Chemical constituents: The leaf extract of *Scoparia dulcis* was claimed by local ethnomedical practitioners to have antidiarrhoea activity especially for the treatment of diarrhoea in children. Phytochemical screening of the leaf extract showed that it contained alkaloids, tannins, saponins, glycosides, proteins and starch. Antimicrobial assay revealed that the extract had the ability of inhibiting gram positive and gram negative bacteria, such as *E. coli, S. aureus, S. feacalis, K. aerogenes* and *P. aeroginosa* but exerted no effect on *C. tropicalis.* Antidiarrhoea assay of the extract on adult albino mice and adult Wister rats induced diarrhoea using castor oil showed that the extract inhibited diarrhoea at the lowest dose of 30 mg/ml/kg body weight (Umeh *et al*. 2012). The phytochemical analysis of the powdered whole plant revealed the presence of carbohydrates, flavonoids, saponins, tannins, alkaloids, steroids and terpenes. Successive extraction yielded 1.93% hexane extract, 1.54% ethyl acetate extract, and 14.50% methanol extract. Quantitative pharmacognostic analysis gave 7.74% moisture content, 20.00% alcohol extractive value, 20.00% water extractive value, 6.32% total ash, 0.82% acid-insoluble ash and 0.37% water-soluble ash (Okhale *et al*. 2010).

1.17 *Senna occidentalis* (L.) Link

Family name: FABACEAE

Voucher number: BKF: 154230

Common Names: Coffeeweed, Senna coffee

Thai names: Khi lek thet (ขี้เหล็กเทศ)

Part used: whole plant

Figure 17 *Senna occidentalis* (L.) Link

Description: Shrub, 1-3 m high. Leaves: paripinnate, alternate; leaflets: 4-9 pairs, lanceolate, 1-2 cm wide, 2-5 cm long; stipules: ovate, caducous. Inflorescence: in axillary, few-flowered corymb; flowers: yellow. Pods: swollen, nearly straight (Saralamp *et al*., 1997).

Traditional uses: Use in the treatment of fever, infected wound and pruritic rash (Pongboonrod, 2493). Applied in the treatment of abscess (Traditional medical school faculty, 2505). In India, it is used for the treatment of abscesses, bites (scorpion), constipation, diabetes, edema, fever, inflammation, itch, liver diseases, liver support, rheumatism, ringworm, scabies, skin diseases, snake bite, wounds. In Mexico, it is used to relieve chills, digestive sluggishness, dyspepsia, earache, eczema, edema, fatigue, fever, headache, inflammation (skin), laxative, leprosy, nausea, pain,

rash, rheumatism, ringworms, skin problems, sores, stomachache, swelling, tumors, ulcers, venereal disease, water retention, worms, yellow fever (Omoregie *et al*., 2010).

Chemical constituents: Ethanol extract contained tannins, saponins, cardiac glycoside, terpenoids and anthraquinones while the fraction revealed the presence of tannins, terpenoid and anthraquinones (Sadiq *et al*. 2012). Leaf extracts were evaluated for antimicrobial activity against six pathogenic strains of gram-positive, gram-negative bacteria (*S. aureus, P. aeruginosa, S. faecium, E. coli, N. gonorrheae, P. vulgarius)* and four different fungal species (*A. niger, A. tamari, F. oxysporum P. oxalicum*). Phytochemical studies revealed the presence of saponins, tannins, alkaloids and flavonoids in the water, acetone and hexane extracts. The plant extracts demonstrated antimicrobial activity against both the tested bacteria and fungi with water extract demonstrating the highest activity followed by the hexane extract (Ettu *et al.* 2011).

1.18 *Sida acuta* Burm.f.

Family name: MALVACEAE

Voucher number: BKF: 154207

Common Names: spiny headed sida, broom weed.

Thai names: Ya khat mon (หญ้าขัดมอน)

Part used: whole plant

Figure 18 *Sida acuta* Burm.f.

Description; A low shrub to 1-2 m tall with slender tough stems; leaves: lanceolate or linear-lanceolate, acute, obtuse or rounded at base, 1.5-6 cm long, 5-20 mm wide, sharply serrate, stellate-pubescent beneath; petioles: 3-5 mm long; blades: 3-nereved from base, pinnately branched above the base; stipules: linear-acute, about 1 cm long; flowers: 1 or 2 in axils, pedicels to about 1 cm long; corolla: about 6-14 mm broad, yellow; fruits: 4-5 mm broad, 6-8-carpellate; seeds: dark reddish-brown, 1.5 mm long; ripe carpels: with 2 beaks, grooved on the back (Stone, 1970).

Traditional uses: Use in the treatment of manifestation of infection (Pongboonrod, 2493). Applied in the treatment of venereal ulcers (Veeraphajuk, 2545), sore throat (Veesapen, 2548a), dysentery severe stage, abscess, tetanus and fever with jaundice (Veesapen, 2548b).

Chemical constituents: The phytochemicals detected were alkaloids, tannins, saponins, reducing compounds, flavonoids, terpenoids, phenolic compounds and glycosids in moderate amounts. The antibacterial activities of crude extracts were against *S. faecalis* and *E. coli.* (Ibrahim *et al*., 2012). Antibacterial and antifungal activity studies of leaf extracts of *Sida acuta* were carried out. The phytochemical tests of extracts revealed the presence of carbohydrates, alkaloids, phytosterols, saponins and fixed oils (Akilandeswari *et al*., 2010).

1.19 *Stephania venosa* (Blume) Sreng.

Family name: MENISPERMACEAE
Voucher number: BKF: 154235
Common Names: Stephania, blood-soap
Thai names: Kra thom lueat (กระท่อมเลือด)
Part used: Bulb

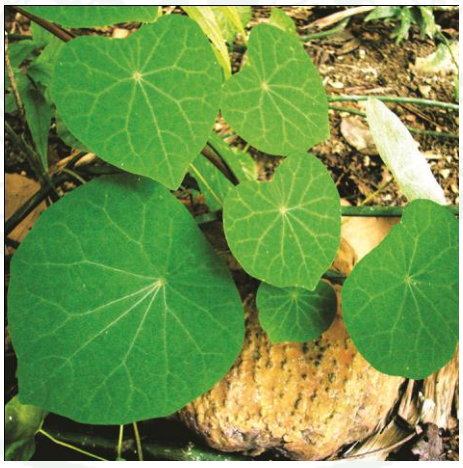

Figure 19 *Stephania venosa* (Blume) Sreng.

Description; Slender climbers, reddish sap; leafy stem herbaceous, annual, arising from a large exposed tuber. Leaves: simple, alternate, broadly triangular-ovate, margin often slightly lobed, 7-12 cm wide, 6-11 cm long, lower surface minutely papillose. Inflorescence: in axillary umbelliform cyme, 4-16 cm long;

flowers: orange. Famale inflorescence is much more condensed then the male. Fruits: drupe, obovate (Saralamp *et al*., 1997).

Traditional uses: Use in the treatment of cancerous or non cancerous chronic ulcers, leukorrhea (Chotianun, 2553), diarrhea (Boonyaphapas and Chokchaipon, 2543), hypersputum and dysentery (Pongboonrod, 2493; Rujjanawate, 2548).

Chemical constituents: (-)-anonaine, (-)-asimilobine, ayuthianine, cepharamine, (-)-crebanine, (-)-4-hydroxy-crebanine, 7-oxo-crebanine, (-)- apo-glaziovine,, kamaline, (-)-kikemanine, liriodenine, (-)-mecambroline, (-)- nuciferoline, (-)-tetrahydro-palmatine, (+)- reticuline, oxo-stephanine, oxo-stephanosine, (+)-stepharine, N –carboxamido (-)-stepharine, 6-stepharinosine, (-)-O-methyl-(-)-stepharinosine, (-)-stesakine, sukhodianine, (-)-sukhodianine, sukhodianine -β-N-oxide, thailandne, (-)-tudoranine (+)-thalirugosamine; (-)-ushinsunine, (-)-ushinsunine -β-N-oxide, ushinsunine -β-N-oxide, (-)-4α-hydroxy, ushinsunine, uthongine (Boonyaphapas and Chokchaipon, 2543).

1.20 *Suregada multiflorum* (A.Juss.) Baill.

Family name: EUPHORBIACEAE

Voucher number: BKF: 154231

Common names: Suregada

Thai name: Khan Thong (ขันทอง)

Part used: roots

Figure 20 *Suregada multiflorum* (A.Juss.) Baill.

Description; Tree is 4-15 cm high. Leaves are simple, alternate, oblong, 3-6 cm wide, 9-14 cm long. Inflorescence is in axillary fascicle, unisexual, dioecious; flowers are yellowish white. Fruits are loculicidal capsule, 3-valved, and subglobose (Saralamp *et al.*, 1996).

Traditional uses: Use in the treatment of skin diseases with pruritus, cancerous or non-cancerous chronic ulcers, worm, gingivitis (Pongboonrod, 2493; Rujjanawate, 2548). Applied in the treatment of abscess (S.S., 2483), carcinoma of hypopharynx and hypopharyngeal cancer (Veesapen, 2548b). Wood is antipyretic. It is used in the treatment of venereal diseases and externally used for urticaria. Stem bark is anthelmintic and laxative. It is externally used as fungicide, as well as apply locally to strengthen gums and teeth (Saralamp *et al.*,1996).

Chemical constituents: caprylic acid, gelomulide A, gelomulide B, gelomulide C, gelomulide D, gelomulide E, gelomulide F, gelomuloside A, gelomuloside B, gelonin, gelonium anti-HIV protein GAP-13, jolkinolide B, luteolin-4'-7-dimethyl ether 3'-O-β-D-glucoside, multiflorenol, myristic acid, oleic acid, palmitic acid, 4'-7-dimethyl: 6-O-β-D-glucoside scutellarein, β-sitosterol (Boonyaphapas and Chokchaipon, 2539).

The methanol: ethyl acetate (1:9) extract of the roots and leaves were tested against five Gram-positive and four Gram-negative bacteria. Root extract was active against all tested microbial species and the highest activity was shown against *E. coli.* Leaf extract showed mild activity for all the tested bacterial strains (range of inhibition zone was 5 ± 0.10 to 6 ± 0.13 mm) except for *B. cereus, B. megatherium, B. anthracis and S. boydii*. Significant and highest minimum inhibitory concentration (MIC) value was observed on the root extract against *E. coli* with the value of 0.625 mg/ml. In insecticidal study, the root extract of *S. multiflora* showed better activity with 100% mortality rate of *Tribolium castaneum* at a dose of 50 mg/ml for 12 hours and also showed the activity in a dose dependent manner, whereas the leaf extract of *S. multiflora* showed 40% mortality rate of *Tribolium castaneum* at a dose of 50mg/ml for 48 hours (Islam *et al.*, 2011).

2. Pathogenic bacteria

Microorganisms used in the determination of antibacterial activities of different plant extracts in this study were *Bacillus cereus*, *Escherichia coli* and *Staphylococcus aureus*.

2.1 *Bacillus cereus*

B. cereus, a Gram-positive bacilli bacterium, causes two different forms of food poisoning, which are an emetic illness and a diarrhoeal illness. It has also been reported to associate with septicemia, respiratory infection, pneumonia, meningitis, wound and ocular infection (Olawale *et al*., 2011). It is a cause of problems in the food industry (Schlegelova *et al*., 2003). It has been reported to develop overall resistance to several antibiotics (Whong and Kwaga, 2007). It is widespread in soil, milk, the surfaces of meat and poultry, cereals, starches, herbs and spices. Its role as a food-borne pathogen is relatively recent and somewhat uncommon in the United States. Two distinct types of illness have been attributed to the consumption of food contaminated with *B. cereus*. The more common manifestation is a diarrheal illness with an incubation time of 8-16 h characterized by abdominal pain and diarrhea. The other is an emetic illness with an incubation time of 1-5 h and characterized by nausea and vomiting. While the emetic type is usually associated with cereal type products such as rice, the diarrheal type is more widely associated with many foods. *B. cereus* typically is a very large, aerobic, Gram-positive, spore-forming rod with peritrichous flagella. It grows over a wide temperature range (10 to 48°C) with an optimum range of 28 to 35°C. It can grow over a wide pH range (pH 4.9 - 9.3) and in sodium chloride concentrations approximating 7.5%. Microscopically it may be seen in chains. Macroscopically the colonies have a dull or frosted appearance on a nutrient agar plate. Its association with diseases is usually related to counts of higher than 10^5 cfu/g in the suspected food. Since *B. cereus* does not ferment mannitol, does produce lecithinase and is resistant to polymyxin, a selective medium consisting of mannitol-yolk-polymyxin (MYP) is commonly used for its isolation. Colonies typically are pink in color and surrounded by a zone of precipitate. An ELISA test is available to

detect the diarrheal toxin (Lattuada and McClain, 1998).

2.2 *Escherichia coli*

E. coli is Gram-negative, rod-shaped bacteria belonging the family Enterobacteriaceae. Sources and prevalence of Enteropathogenic *E. coli* (EPEC) are controversial because foodborne outbreaks are sporadic. Humans, bovines, and swine can be infected, and the latter often serve as common experimental animal models. *E. coli* is present in the normal gut flora of these mammals (Walderhaug, 1992). *E. coli* is a common component of the aerobic bowel flora and causes urinary, wound, lung, meningeal and septicaemic infections. Some strains are important causes of travellers' diarrhoea and the haemolyic-uraemic syndrome. *E. coli* is normal commensal flora that must be distinguished from intestinal pathogens. Its presence in water supplies indicates evidence of faecal contamination. Enteropathogenic *E. coli* strains are classified by their O antigens (and subdivided by H and K antigens). Important strains are Enteroadhesive (EAEC), Enteroinvasive (EIEC), Enterotoxigenic (ETEC) and Enterohaemorrhagic (EHEC) (Spicer, 2008). The urinary tract infection is complicated by the increasing incidence of infections caused by various strains of *E. coli* resistant to commonly used antimicrobial agents (Manges *et al*., 2001). *E. coli* is sometimes used as a sentinel for monitoring antimicrobial drug resistance in fecal bacteria because it is found more frequently in a wide range of hosts, acquires resistance easily, and is a reliable indicator of resistance in salmonellae (Tadesse *et al*., 2012).

2.3 *Staphylococcus aureus*

S. aureus is a Gram-positive, spherical bacterium (coccus) which on microscopic examination appears in pairs, short chains, or bunched, grape-like clusters. Some strains are capable of producing a highly heat-stable protein toxin that causes illness in humans (Walderhaug, 1992). *S. aureus*, which produces the enzyme coagulase and usually has golden-yellow colonies, is the major human pathogen. It is pyogenic (pus-producing), causing abscesses in skin and most other organs, leading to

bacteraemia and endocarditis, and also produces many toxins (Spicer, 2008). Incidence of *S. aureus* infection has increased during the past two decades. Bacteremia due to *S. aureus* has been reported to be associated with mortality rates of 15–60% (Cosgrove *et al*., 2003). *S. aureus* is among the most prevalent causes of clinical infections globally and has garnered substantial public attention due to increasing mortality associated with multi-drug resistance (Waters *et al*. 2011).

The onset of symptoms in staphylococcal food poisoning is usually rapid and in many cases acute, depending on individual susceptibility to the toxin, the amount of contaminated food eaten, the amount of toxin in the food ingested, and the general health of the victim. The most common symptoms are nausea, vomiting, retching, abdominal cramping, and prostration. Some individuals may not always demonstrate all the symptoms associated with the illness. In more severe cases, headache, muscle cramping, and transient changes in blood pressure and pulse rate may occur. Recovery generally takes two days. However, it is not unusual for complete recovery to take three days and sometimes longer in severe cases. A toxin dose of less than 1.0 microgram in contaminated food will produce symptoms of staphylococcal intoxication. This toxin level is reached when *S. aureus* populations exceed 10^5cfu per gram (Walderhaug, 1992).

3. Infectious Diseases

3.1 Lung abscesses

Lung abscesses are pus-containing cavities within the lung; they may be single or multiple, uni- or bilateral. Infection can be introduced via five routes. The commonest route is from the air passages above the lungs, i.e. through the bronchi by inhalation (from sinusitis, dental or oral sepsis, bronchitis or bronchiectasis) or by aspiration (lost gag/swallowing reflexes, coma or anaesthesia, oesophageal reflux, foreign body or tumour). In this case, microorganisms usually found in hospitalized pateints include oro-pharyngeal flora of mixed anaerobes and mixed streptococci, plus gram-negative rods. The second route of infection comes from organs below the

lungs. In this case, mixed enteric flora including anaerobes and aerobic gram-negative rods and cocci, as well as *Entamoaba histolytica*, *Paragonimus westermani*, causing paragonimiasis are found. The third infection route is from within the lungs, following pneumonia. Microorganisms associated with this case include *Klebsiella pneumonia* and other Gram-negative rods and *S. aureus*. The presence of *Legionella* spp., *Actinomyces* and *Nocardia* spp. are rare. *Pseudomonas pseudomallei*, causing melioidosis, is also found. The fourth route of infection is from external organs. Microorganisms found include *S. aureus*, skin flora, soil organisms. The last route of infection is from remote sources. Microorganisms associated in this case include *S. aureus*, *E. coli*, and anaerobes, especially *Fusobacterium necrophorum* and *Bacteroides fragilis* (Spicer, 2008).

3.2 Diarrhoea (Spicer, 2008)

A number of clinical syndromes arise from ingestion of pathogens. These can be confined to the gut or can spread to cause, e.g., liver and intra-abdominal abscesses. The clinical features can be grouped according to the pathogenesis.

3.2.1 *B. cereus*

B. cereus causes either short-incubation emetic illness from ingestion of pre-formed toxin, usually in rice, but also meats or vegetables, or longer-incubation diarrhoeal illness when ingested *B. cereus* produces toxin in the gut. Both illnesses are short in duration.

3.2.2 *E. coli*

E. coli is a member of normal gut flora; some entero-pathogenic strains (EPEC) have virulence factors causing diarrhoea and other gut damage. Enterohaemorrhagic *E. coli* (EHEC) strains produce verotoxin, which binds to receptors on gut mucosa and the kidney causing mucosal damage and haemorrhage. Serotype O157 causes haemorrhagic colitis (HC) and the haemolytic-uraemic

syndrome (HUS) of haemolytic anaemia, thrombocytopenia and acute renal failure. Enteroinvasive *E. coli* (EIEC) strains invade gut mucosa, multiply, spread and destroy it, causing bloody diarrhoea. Enteroadhesive *E. coli* (EAEC) adhere to and destroy the gut microvilli. Enterotoxigenic *E. coli* (ETEC) strains produce a heat-labile enterotoxin (LT) similar to cholera toxin, and/or several heat-stable toxins (STs). They cause fluid secretion and hence diarrhea which is the commonest cause in children in developing countries.

3.2.3 *S. aureus*

S. aureus causes sudden vomiting within 2-8 hours through pre-formed toxin, very similar to the emetic form of *B. cereus* disease.

3.3 Cystitis

The bladder is usually infected from the perineum and urethra, especially when sexual activity or poor perineal hygiene causes fecal contamination. As most uro-pathogens come from fecal flora, the commonest is *E. coli*, causing 80% of community-acquired and 40% of hospital-acquired infections (Spicer, 2008).

3.4 Intrarenal abscess

Renal abscesses are characterized by collections of pus within kidney tissue. Ascending infection is the commonest mechanism, causing pyelonephritis especially with obstruction of urine flow; this usually also causes multiple abscesses. Haematogenous infection in bacteraemia is also usually multiple. Infection of a renal cyst, by bacteraemia or an operative procedure, is usually single. Ascending infection is by the usual uro-pathogens, i.e. *E. coli, Klebsiella* spp. and other fecal pathogens. Haematogenous or operative infection is often with *S. aureus* (Spicer, 2008).

3.5 Furuncles (boils)

Boils are a common and more severe form of folliculitis, often affecting sebaceous and sweat glands. A boil is a painful, tender, elevated red-rimmed pustule that grows until it discharges or is opened with a sterile needle or blade. There is usually no host abnormality apart from nasal carriage of the causative strain of *S. aureus*. In contrast, there is often some host abnormality such as acne, diabetes or abnormal white cell function. They can be classified as furunculosis (multiple boils), chronic furunculosis (persistent or recurrent boils), and hidradenitis suppurativa (a deep suppurative infection of the sweat glands, usually in axilla or groin). *S. aureus* and *Streptococcus pyogenes* are easily the commonest causes of cellulitis; less often, group B, C or G streptococci, *Aeromonas hydrophila* from fresh water, *Erysipelothrix rhusiopathiae* from meat or fish, enteric Gram-negative rods, or other organisms are responsible (Spicer, 2008).

3.6 Toxic shock syndrome (TSS)

TSS is a serious systemic infection caused by *S. aureus* strains producing toxic shock syndrome toxin 1 (TSST-1). Predisposing factors include multiplication of the organisms and magnesium binding in certain high-absorbency tampons and a genetically ineffective antibody response. Skin or other tissues may be the primary site in men and nonmenstruating women (Spicer, 2008).

4. Antibiotics

Antibiotics are chemical substances produced from various microorganisms (bacterial and fungus) that kill or suppress the growth of other microorganisms. The term is also used for synthetic antimicrobial agents such as sulfonamides and quinolones (Salerno, 1999).

4.1 Mechanisms of action of clinically used antimicrobial.

Good antibiotics are those that exhibit selective toxicity. Such drugs are harmful only to pathogenic microorganisms without being harmful to the host. The mechanisms of action can be placed under four headings:

4.1.1 Inhibition of cell wall synthesis.
4.1.2 Inhibition of cell membrane function.
4.1.3 Inhibition of protein synthesis (ie, inhibition of translation and transcription of genetic material)
4.1.4 Inhibition of nucleic acid synthesis (Brooks *et al*., 2010)

5. Major groups of antimicrobial compounds from plants

Plants are rich in wide variety of secondary metabolites. Useful antimicrobial phytochemicals can be divided into several categories (Cowan, 1999).

5.1 Phenolics and polyphenols

5.1.1 Simple phenols and phenolic acid

Most of the simple phenols are monomeric components of the polymeric polyphenols and acids which make up plant tissues, including lignin, melanin, flavolan and tannins. The sites and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity. The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins.

Phenolic compounds possessing a C3 side chain at a lower level of oxidation and containing no oxygen are classified as essential oils and often cited as

antimicrobial as well.

5.1.2 Quinones

Quinones are aromatic rings with two ketones substitution, typically form colored pigments covering the entire visible spectrum. Generally, they are derived from benzoquinone, naphthoquinone or anthroquinone structures. Propable targets in the microbial cell are surface-exposed adhesins, cell wall polypeptides and membrane-bound enzymes.

5.1.3 Flavones

Flavones are phenolic structures containing one carbonyl group. The flavonoids have two benzene rings separated by a propane unit and are derived from flavone. They are generally found in plants as their glycosides. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. More lipophilic flavonoids may also disrupt microbial membrane.

5.1.4 Tannins

Tannins are common to vascular plants existing primarily within woody tissues. Plant tissues that are high in tannin content have a highly bitter taste and are avoided by most feeders. Previous report reviewed the antimicrobial properties of tannins. According to these studies tannin can be toxic to filamentous fungi, yeast and bacteria. Their mode of antimicrobial action may related to their ability to inactivate microbial adhesins, enzymes, cell envelop transport proteins, etc.

5.1.5 Coumarin

Coumarins are phenolic substance made of fused benzene and ∝-pyrone rings. They are responsible for the characteristic odor of hay. Coumarin is

found *in vitro* to inhibit *Candida albicans*. They have been found to stimulate macrophages, which could have an indirect negative effect on infection.

5.2 Terpenoids and Essential Oils

The oils are secondary metabolites, which are highly enriched in compounds based on an isoprene structure. They are called terpenes. Their general chemical structure is $C_{10}H_{16}$, and they occur as diterpenes, triterpenes, and tetraterpenes (C20, C30 and C40), as well as hemiterpenes (C5) and sesquiterpenes (C15). When the compounds contain additional elements usually oxygen, they are termed terpenoids. Terpenes or terpenoids are active against bacteria, viruses and protozoa. The mechanism of action of terpenes is not fully understood but it speculated to involve membrane disruption by the lipophilic compounds.

5.3 Alkaloids

Heterocyclic nitrogen compounds are called alkaloids. The mechanism of action of highly aromatic planar quaternary alkaloids is attributed to their ability to intercalate with DNA.

5.4 Lectins and polypeptides

Peptides are often positively charged and contain disulfide bonds. Their mechanism of action may be the formation of ion channels in the microbial membrane or competitive inhibition of adhesion of microbial proteins to host polysaccharide receptors.

MATERIALS AND METHODS

EXPERIMENT Part 1: To investigate antibacterial activities of twenty medicinal plants.

1. Plant materials

Laboratory study on plant extracts was conducted at Plant Natural Products Laboratory, Faculty of Agriculture Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus. Study of antimicrobial activity of Thai medicinal plants was conducted at Central Laboratory and Green House Complex, Kasetsart University, Kamphaeng Saen Campus. Twenty plant materials were collected from Udon Thani and Kanchanaburi provinces, Thailand. Herbarium specimens of all twenty plants were deposited at The Forest Herbarium (BKF) at the Nation Park, Wildlife and Plant Conservation Department, Bangkok, Thailand. The voucher specimen numbers of the plants, species, family and parts used are shown in Table 1.

Table 1 List of twenty medicinal plants used in the experiment and their traditional uses

Species	Family	Voucher number	Parts used
Albizia chinensis (Osbeck) Merr.	Mimosaceae	BKF:154214	roots
Catunaregam tomentosa (Blume ex DC.) Tirveng.	Rubiaceae	BKF:154226	roots
Cleome viscosa L.	Capparidaceae	BKF:154215	whole plant
Clerodendrum inerme (L.) Gaertn.	Verbenaceae	BKF:154225	roots
Combretum qurdrangulare Kurz	Comberetaceae	BKF:154216	roots
Dioecrescis erythroclada (Kurz) Tiveng.	Rubiaceae	BKF:154234	roots
Diospyros mollis Griff.	Ebenaceae	BKF:154210	roots
Ixora javanica (Blume) DC.	Rubiaceae	BKF:154213	roots
Lepisanthes senegalensis (Poir.) Leenh.	Sapindaceae	BKF:154229	roots
Luvunga scandens (Roxb.) Buch.-Ham.	Rutaceae	BKF:154228	roots
Pavetta indica L.	Rubiaceae	BKF:154219	roots
Phyllanthus emblica L.	Euphorbiaceae	BKF:154222	roots
Polyalthia cerasoides (Roxb.) Benth. ex Bedd.	Annonaceae	BKF:154232	roots
Pouzolzia hirta (Blume) Hassk.	Urticaceae	BKF:154220	whole plant
Psidium guajava L.	Myrtaceae	BKF:154223	leaves
Scoparia dulcis L.	Scrophulariaceae	BKF:154209	whole plant
Senna occidentalis (L.) Link	Fabaceae	BKF:154230	whole plant
Sida acuta Burm.f.	Malvaceae	BKF:154207	whole plant
Stephania venosa (Blume) Sreng.	Menispermaceae	BKF:154235	bulb
Suregada multiflorum (A.Juss.) Baill.	Euphorbiaceae	BKF:154231	roots

2. Chemicals & Equipments

2.1 Mueller-Hinton Agar plates
2.2 Autoclave
2.3 Analytical balance (± 0.0001 g accuracy)
2.4 Petri dishes (Pyrex)
2.5 Centrifuge
2.6 Cylinders
2.7 Electric grinder
2.8 Erlenmeyer flasks
2.9 Filter cups
2.10 Filter papers
2.11 Hot air oven
2.12 Incubator
2.13 Paper discs
2.14 Pipettes
2.15 Centrifuge tubes (Pyrex)
2.16 Rotary evaporator (Buchi Vacuum pump, Buchi)
2.17 Round-bottom flasks

3. Plant extracts

The plants were air-dried under the shade at room temperature and cut into small pieces before grinding into fine powder with an electric grinder. The ground materials were stored in airtight containers protected from light. The plant powders were extracted with 95% ethanol and hexane. Two hundred and fifty grams of each plant powder was mixed with 2 L of each solvent. The mixtures were left for 7 days at room temperature and then filtered through Whatman No.1 filter papers. The mixtures were re-extracted 2 times at 7-day interval with 2 L of sovent each time. The extracts were concentrated to dryness by a rotary evaporator at 50^{o}C under reduced pressure to obtain crude extract. The crude extracts were weighed and recorded and then stored for further antibacterial tests.

4. Antibacterial assays

4.1 Bacterial strains

Bacillus cereus KB70, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used in the determination of antibacterial activities of different plant extracts. All bacterial strains were obtained from Central Laboratory and Green House Complex, Kasetsart University. The bacterial strains were maintained on nutrient agar media (Collee and Marr, 1989) and subcultures were freshly prepared before use. Bacterial cultures were prepared by transferring two to three colonies into a tube containing 5 ml nutrient broth and grown overnight at 37^{o}C. The turbidity of the culture was adjusted with sterile saline solution to match 0.5 McFarland standard.

4.2 Disc diffusion assay

4.2.1 Antibacterial screening

Antibacterial activities of the 20 plant extracts were evaluated using disc diffusion method according to the M2-A8 Document guidelines on Mueller-Hinton agar plates (NCCLS, 2003b). Sterile cotton swabs were dipped into the standard bacterial strain suspensions (0.5 McFarland, containing 1.5 x 10^{8}cfu/ml) before inoculating evenly onto the entire surface of the agar plates. Plant extract solutions at different concentrations were prepared by dissolving the dried extracts obtained from the previous step in DMSO. All plant extract solutions were sterilized by Minisart® syringe filter (0.45 µm pore size; Sartorius, Germany) prior use. Sterile paper discs (6 mm diameter) were impregnated with 20 µl of the sterile plant extract solutions. The paper discs, containing 0, 0.625, 1.25, 2.5, 5 and 10 mg of each extract/disc, were applied onto the inoculated agar plates. A paper disc containing only DMSO was used as a negative control. The plates were incubated at 37^{o}C for 24 hours. Clear zone around the paper discs, indicating the inhibition of bacterial growth, were measured and the average diameter of the clear zones were interpreted as

antibacterial activity of the extracts according to Ruengsakul (1987). The experiment was performed in triplicate. The extracts which had high antibacterial activities were subsequently determined for their minimum inhibitory concentrations (MIC).

5. Statistical analysis

For data on disc diffusion assays, independent sample t-test (SPSS program) was used to determine the significant difference ($P < 0.05$) in antimicrobial activities between the ethanol and hexane extract of each plant.

EXPERIMENT Part 2: To determine antibacterial potency of selected medicinal plant extracts.

This experiment was conducted at Central Laboratory and Green House Complex, Kasetsart University, Kamphaeng Saen Campus under the supervision of Dr. Manee Tantirungkij and her staff.

1. Plant materials

The results from the screening steps in the Experiment 1 showed the samples with high potential in inhibiting the growths of all three bacterial strains included the ethanol and hexane extracts obtained from *C. inerme, C. qurdrangulare, P. guajava* and *S. venosa*. Therefore, these extracts were selected for the determination of their minimum inhibitory concentrations (MICs).

2. Antibacterial assays

2.1 Bacterial strains

Bacillus cereus KB70, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used in the determination of antibacterial activities of different plant extracts. All bacterial strains were obtained from Central Laboratory and Green House Complex, Kasetsart University. The bacterial strains were maintained on different nutrient agar media (Collee and Marr, 1989) and subcultures were freshly prepared before use. Bacterial cultures were prepared by transferring two to three colonies into a tube containing 20 ml nutrient broth and grown overnight at 37^{o}C. The turbidity of the culture was adjusted with sterile saline solution to match 0.5 McFarland standard.

2.2 Minimum Inhibitory Concentration (MIC) determination

The MIC endpoint was the lowest extract concentration that showed

absence of growth or complete growth inhibition (100% inhibition). MICs of the extracts with high antibacterial activities were carried out in the same manner as described previously (NCCLS, 2003b). Sterile paper discs (6 mm diameter) containing 0, 0.009, 0.019, 0.038, 0.078, 0.156, 0.312, 0.625, 1.25, 2.5, 5 and 10 mg of each extract/disc were placed onto the inoculated agar plates before incubating at 37^{o}C for 24 hours. The extract exhibited the highest antibacterial activity was further investigated by broth dilution assay.

2.3 Broth dilution assay

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of the selected extract which exhibited the highest antibacterial activity were determined by broth dilution method (NCCLS, 2003a).

2.3.1 Determination of MIC

Each microdilution well containing 50 µl of serially two-fold diluted extract concentration was inoculated with 50 µl of diluted bacterial suspension (0.5×10^6 cfu/ml) in 2x Mueller-Hinton broth. The microdilution trays were incubated at 37^{o}C for 24 hours. Turbidity of cell growth was measured by ELISA reader at 595 nm. The MIC endpoints were the lowest extract concentrations that showed absence of growth or complete growth inhibition (100% inhibition).

2.3.2 Determination of MBC

MBCs of the antibacterial crude extracts were determined by subculturing 5 µl of the 24-hour culture from each well of microdilution plates used for MIC determination onto Mueller-Hinton agar plates. The plates were incubated at 37^{o}C until growth was seen in the growth control subculture (usually before 48 h). The MBC was the lowest extract concentration that showed either no growth or fewer than three colonies to obtain approximately 99 to 99.5% killing activity.

EXPERIMENT Part 3: To screen the pharmacognostic characteristics and phytochemicals of *Clerodendrum inerme* (L.) Gaertn. roots.

This experiment was conducted at Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University under the supervision of Associate Professor Dr. Wanee Gritsanapan and her staff.

1. Plant material

The results from the screening steps in the Experiment 2 showed the samples with the highest potential in inhibiting the growths of all three bacterial strains was the ethanol extract of *Clerodendrum inerme* (L.) Gaertn. roots. Therefore, this extract was selected for screening the pharmacognostic characteristics and phytochemicals.

2. Identification

2.1 Pharmacognostic characteristics

2.1.1 Microscopic: Fresh sample of the roots of *C. inerme* was dried at 60°C then powdered by an electronic mill. The powder was passed through a sieve no. 60 to yield fine powder. The powder was kept in a tightly-closed vial until used.

2.1.2 Powdered sample: The powder was creamish brown, had a characteristic odour and was tasteless. The characteristics of the *C. inerme* root powder were examined under a microscope (Olympus, Japan) using mounting reagents. The characteristic tissues were drawn using a camera lucida (Olympus, Japan).

3. Chemical identification tests

3.1 An extraction was performed by mixing 500 mg of the test sample with 10 ml of hot water in a small flask. The mixture was shaken well for 1-2 min, then filtered. To 1 ml of the filtrate in a test tube, 9 ml of distilled water was added, and the mixture was shaken vigourously for 10 sec. The formation of a characteristic honeycomb froth, which persisted for at least 30 min after shaking, was an indication of the presence of saponins.

3.2 One gram of the test sample was refluxed with 30 ml of 80% ethanol for 15 min and filtered. To 2 ml of the filtrate, a few drops of freshly prepared 1% w/v ferric chloride solution was added. A positive result for the presence of phenolic compunds was indicated by the formation of a deep green-blue color.

4. Confirmation test by thin layer chromatography (TLC)

TLC of the extract sample was carried out on an aluminium precoated silica gel 60 F254 plate (Merck) using dichloromethane:methanol (9:1) as a mobile phase.

The test solution was prepared by warming 300 mg of the test sample with 5 ml of methanol on a hot water bath for 2 min. The mixture was left to cool, and then filtered. The residue was washed with sufficient amount of methanol until 5 ml filtrate was obtained.

5. Determination of ash contents (ASEAN Countries, 1993)

5.1 Total ash content

Two to four grams of the test sample was weighed into a tared crucible. The material was incinerated by gradually increasing the temperature, not exceeding 450^{o}C until free from carbon, then it was cooled in a desiccator and weighed. If a carbon-free ash could not be obtained in this way, the charred mass was mixed with

hot water then filtered through an ashless filter paper. The insoluble residue and the filter paper were incinerated until the ash was white or nearly so. Then the filtrate was added to the ash before evaporation to dryness. The sample was heated until its color completely changed to dull redness. The residue was cooled, weighed and calculated the percentage of total ash with reference to the air-dried crude sample.

5.2 Acid-insoluble ash content

To the crucible containing the residue obtained from the determination of total ash, 25 ml of hydrochloric acid (2 N) was added. The crucible was covered with a watch-glass and boiled gently for 5 min. The watch-glass was rinsed with 5 ml of hot water and the rinsing water was added to the crucible. The insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight. The percentage of acid-insoluble ash was calculated with reference of the air-dried crude sample.

6. Determination of loss on drying (ASEAN Countries, 1993)

The sample (5g) was accurately weighed into a tared flat weighing bottle, dried in the oven at about 100-105^{o}C for about 5 h and then weighed. The drying and weighing were continued at 1-h intervals until the constant weight was obtained. The percentage of loss on drying was calculated with reference to the air-dried crude sample.

7. Determination of soluble extractives (ASEAN Countries, 1993)

7.1 Water soluble extractives

The air-dried sample (4 g) was accurately weighed, and coarsely powdered into a suitable glass-stoppered flask. The sample was macerated with 100 ml of chloroform-water (2.5 ml of chloroform in sufficient amount of water to make 1000

ml) for 24 h. The mixture was shaken frequently during the first 6 h and allowed to stand for 18 h. Then it was filtered rapidly with precautions on loss of solvent. The filtrate (25ml) was evaporated to dryness in a tared flat-bottomed dish on a water-bath and dried at 105^{o}C for 6 h. the dish was cooled in a desiccator before accurately weighed. The percentage of extractives was calculated with reference to the air-dried crude sample.

7.2 Ethanol soluble extractives

The air-dried sample (4 g) was accurately weighed before coarsely powdered into a suitable glass-stoppered flask. The sample was macerated with 100 ml of ethanol for 24 h. The mixture was shaken frequently during the first 6 h and allowed to stand for 18 h. It was then rapidly filtered with precautions on loss of solvent. The filtrate (25 ml) was evaporated to dryness in a tared flat-bottomed dish on a water-bath and dried at 105 °C for 6 h before cooling in a desiccator. The residue was accurately weighed and the percentage of extractives was calculated with reference to the air-dried crude sample.

7.3 Diluted ethanol (70%) soluble extractives

The air-dried sample (4 g) was accurately weighed, and coarsely powdered into a suitable glass-stoppered flask. The sample was macerated with 100 ml of diluted ethanol (70% v/v) for 24 h. The mixture was shaken frequently during the first 6 h and allowed to stand for 18 h. Then it was filtered rapidly with precautions on loss of solvent. The filtrate (25 ml) was evaporated to dryness in a tared flat-bottomed dish on a water-bath and dried at 105°C for 6 h, before leaving to cool in a desiccator. The residue was accurately weighed and the percentage of extractives was calculated with reference to the air-dried crude sample.

8. Phytochemical screening of *C. inerme* roots (Farnsworth, 1966; Luanratana, 1993)

8.1 Test for alkaloids

Plant extract was prepared by heating 2-4 g of the powdered sample with 5% HCl for 10 min, followed by filtration. A few drops of this acidulated extract were then tested with several alkaloidal precipitating reagents such as Mayer's reagent, Dragendorff reagent, Hager reagent, Marme reagent, Tannic acid, Valser reagent or Wagner reagent to ascertain the presence of alkaloids. A rating of 0, or +1 to +4 on the lack of, or degree of precipitation following use of the reagents were recorded. Another test for alkaloids was that the filtrate was made basic and extracted with an organic solvent (usually chloroform or ether). The organic solvent extract was concentrated and spotted on a filter paper. The Dragendorff spraying reagent was sprayed onto the spot of the sample. Positive of alkaloids was shown by deep orange on the spot.

8.2 Test for flavonoids

Cyanidin reaction detects compounds having the γ-benzopyrone nucleus. To an alcoholic solution of plant material, a small piece of magnesium ribbon was added, and followed by dropwise addition of concentrated HCl. Colors ranging from orange to red (flavones), red to crimson (flavonols), crimson to magenta (flavanones) and occasionally to green or blue were taken as evidence for a positive reaction for either the aglycone or heteroside. Colors usually developed within 1-2 min following addition of the acid and were subjected to the variation in intensity depending on the concentration of flavonoid present in the sample.

8.3 Test for polyphenolic compounds

A ferric chloride solution was added to the ethanol extract of the plant sample resulted in a blue, blue-black, green, or blue-green colour and precipitate, if polyphenolic compounds were present.

8.4 Test for tannins

Tannins are detected most simply in plant extract by the use of gelatin-salt block test. This test employs aqueous extracts prepared from 80% ethanol extracted plant material. A sodium chloride solution was added to one portion of the test extract, 1% gelatin solution to a second portion, and the gelatin-salt reagent to a third portion. Precipitation with the latter reagent, or with both the gelatin and gelatin-salt reagents was indicative of the presence of tannins. If precipitation was observed only with the salt solution (control), a false-positive test was indicated.

8.5 Test for anthraquinones

Modified Borntrager's test was used to detect anthraquinone compounds in the plant sample. The powdered sample (0.3g) was boiled for a few minutes with 0.5 *N* KOH (10 ml) to which was added 1 ml of dilute hydrogen peroxide solution. After cooling, the mixture was filtered. The filtrate (5 ml) was acidified with 10 drops of acetic acid. This acidulated mixture was then extracted by shaking with 10 ml of benzene in a separator and the benzene layer took on a yellow color. A 5-ml sample of the benzene extract was shaken with 2.5 ml of ammonium hydroxide. A positive reaction for the presence of anthraquinones was evidenced by the formation of a red color in the alkaline layer.

8.6 Test for lactones (coumarins)

Coumarins and related volatile compounds in the plant material were detected by a photo effect, by placing a small amount of moistened sample in a test

tube and covering the tube with filter paper moistened with dilute sodium hydroxide solution. The covered test tube was then placed in a boiling water bath for several minutes. Then the paper was removed and exposed to ultraviolet light. If volatile lactone compounds were present, fluorescence appeared within a few minutes. To detect non-volatile lactones, ethanol extract of the plant material in the same test tube was spotted onto a filter paper, to which 1-2 drops of dilute sodium hydroxide solution was added before exposure to ultraviolet light. The fluorescence appeared within a few minutes indicating the present of non-volatile lactones.

8.7 Test for cyanogenetic glycosides

Grignard test was used for detection of cyanogenetic glycosides in the plant sample. The test was conducted by placing about 2 g of moist, shredded plant material in a small test tube, followed by addition of 4 drops of chloroform (to enhance enzyme activity). Sodium picrate solution (5g Na_2CO_3 and 0.5 g picric acid in 100 ml aqueous solution) was prepared. A strip of filter paper was saturated with the solution. The strip then were blotted dry and inserted between a split cork stopper which was then introduced into the neck of the test tube containing the reaction mixture. Care was exercised to ensure that the paper strip did not touch the inner sides of the test tube. The test tube and contents were then warmed at 30-35^{o}C for up to 3 h. High concentration of HCN was detected within 15 min as evidenced by a change in color of the yellow picrate test paper to various shades of red. Absence of a red colour after 3 h was taken as a negative result.

8.8 Test for saponins

Froth test for detection of saponins was used. It is simple and rapid but it can not differentiate triterpenoid from steroidal saponins. The appearance of a characteristic honeycomb froth, which persisted for at least 30 min after shaking an aqueous boiled (3-5 min) mixture containing the plant sample, was presumptive evidence for the presence of saponins.

EXPERIMENT Part 4: To develop a *Clerodendrum inerme* antibacterial gel.

This experiment was conducted at Central Laboratory and Green House Complex, Kasetsart University, Kamphaeng Saen Campus under the supervision of Dr. Manee Tantirungkij and her staff.

1. *Clerodendrum inerme* crude extract

Roots of *Clerodendrum inerme* (L.) Gaertn., Verbenaceae, BKF:154225 was collected from Wutithamwech herbal garden in Kanchanaburi province. The plants were air-dried under the shade at room temperature and cut into small pieces before grinding into fine powder. The plant powder was extracted with 95% ethanol. Two hundred and fifty grams of the plant powder was mixed with 2 L of 95% ethanol. The mixtures were left for 21 days at room temperature and then filtered. The extracts were concentrated to dryness by a Buchi rotary evaporator at 50^{o}C under reduced pressure to obtain crude extract. The crude extract was stored for developing a *Clerodendrum inerme* antibacterial gel.

2. Preparation of hydrogels

Carbopols Ultrez 10, germaben and triethanolamine were obtained from Nhamseang co., Ltd., Bangkok, Thailand. In brief, the gels were prepared by the following procedure (Skalko *et al.*, 1998): Carbopol resin (10 g) was dispersed in distilled water (970 g). The mixture was stirred until thicken and then neutralized by drop-wise addition of triethylamine until a transparent gel was obtained. Quantity of triethylamine was adjusted to achieve desirable gel's consistency. Germaben II (10 g) was added as a preservative. Gels were stored at room temperature to stabilize for 24 hours.

3. *Clerodendrum inerme* antibacterial gel preparation

Ethanol crude extract of *C. inerme* roots was dissolved in DMSO and was added into the hydrogel. Antibacterial gel samples containing 1, 2, 5, and 0 mg of crude extract per 100 ml gel were prepared. Bacterial killing activities of the *C. inerme* gels were then determined.

4. Time kill assays

4.1 Bacterial strains

Bacillus cereus KB70, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used in the determination of antibacterial activities of the gels. All bacterial strains were obtained from Central Laboratory and Green House Complex, Kasetsart University. The bacterial strains were maintained on nutrient agar (Collee and Marr, 1989) and subcultures were freshly prepared before use. Bacterial cultures were prepared by transferring two to three colonies into a test tube containing 5 ml nutrient broth and grown overnight at 37^{o}C. The turbidity of the culture was adjusted with sterile saline solution to match 0.5 McFarland standard.

4.2 Bacterial killing assays

For time-kill study, antibacterial gels containing 1, 2, 5, and 0 mg of crude extract/100 ml gel were tested against *B. cereus*, *E. coli*, and *S. auresus*. The gel samples were placed in test tubes and microbial culture was inoculated to a final concentration at 5 x 10^5 cfu/ml. The mixtures were mixed and left at room temperature. At 0, 3, and 30 min of exposure time, a sample was taken and spreaded on Mueller-Hinton agar plates. The plates were incubated at 37^{o}C for 24 h, then the number of bacterial cells were counted and recorded. This experiment was performed in duplicate.

Time-kill assays were analyzed by determining the number of bacteria which showed decreases in viable count of 1, 2, and 3 $\log_{10}$ cfu/ml compared to the counts at 0 min. Drugs were considered bacteriostatic if they yielded a decrease in the count of ≥ 3 $\log_{10}$ cfu/ml compared to that at 0 min. With the sensitivity threshold (250 cfu/ml) and inocula used, bactericidal activity (99.9% killing and a decrease in the count of ≥ 3 $\log_{10}$ cfu/ml) could be determined. Bacterial carry over was minimized by dilution.

RESULTS AND DISCUSSION

Part 1: To investigate antibacterial activities of twenty medicinal plants.

1. Screening of antibacterial activities of twenty medicinal plants

Table 2 illustrates the differences of antibacterial activities between the ethanol and hexane extracts of twenty medicinal plants against *B. cereus, E. coli* and *S. aureus.* Significant differences ($P < 0.05$) in antibacterial activities were observed between the ethanol and hexane extracts of *Psidium guajava* and *Stephania venosa*. However, it was found that both the ethanol and hexane extracts obtained from *Dioecrescis erythroclada, Lepisanthes senegalensis, Pouzolzia hirta, Scroparia dulcis, Senna occidentalis, Sida acuta* and *Suregada multiflorum* did not exhibit antibacterial activity against the tested bacteria.

Table 2 Antibacterial activities of the ethanol and hexane extracts of twenty medicinal plants at a concentration of 5 mg extract/disc using disc diffusion assay

Sample	Zone of inhibition (mm)								
	B. cereus			*E. coli*			*S. aureus*		
	Ethanol	Hexane	P<0.05	Ethanol	Hexane	P<0.05	Ethanol	Hexane	P<0.05
Albizia chinensis	< 6.0	< 6.0		10.0	9.0		< 6.0	8.5	*
Catunaregam tomentosa	8.0	< 6.0	*	< 6.0	< 6.0		< 6.0	< 6.0	
Cleome viscosa	6.5	6.5		< 6.0	< 6.0		8.0	7.0	
Clerodendrum inerme	11.0	8.0		11.5	< 6.0	*	10.5	8.0	
Combretum qurdrangulare	8.5	7.0		19.5	< 6.0	*	8.0	7.0	
Dioecrescis erythroclada	< 6.0	< 6.0		< 6.0	< 6.0		< 6.0	< 6.0	
Diospyros mollis	8.0	< 6.0	*	< 6.0	< 6.0		8.0	8.0	
Ixora javanica	7.0	8.0		< 6.0	< 6.0		8.0	18.0	*
Lepisanthes senegalensis	< 6.0	< 6.0		< 6.0	< 6.0		< 6.0	< 6.0	
Luvunga scandens	9.0	6.5	*	< 6.0	< 6.0		7.0	8.0	
Pavetta indica	7.0	6.5		< 6.0	< 6.0		7.0	7.0	
Phyllanthus emblica	7.0	< 6.0	*	10.0	9.0		< 6.0	< 6.0	

Table 2 (Continued)

Sample	Zone of inhibition (mm)								
	B. cereus			*E. coli*			*S. aureus*		
	Ethanol	Hexane	P<0.05	Ethanol	Hexane	P<0.05	Ethanol	Hexane	P<0.05
Polyalthia cerasoides	< 6.0	< 6.0		12.0	< 6.0	*	< 6.0	< 6.0	
Pouzolzia hirta	< 6.0	< 6.0		< 6.0	< 6.0		< 6.0	< 6.0	
Psidium guajava	9.5	< 6.0	*	13.5	< 6.0	*	12.0	< 6.0	*
Scroparia dulcis	< 6.0	< 6.0		< 6.0	< 6.0		< 6.0	< 6.0	
Senna occidentalis	< 6.0	< 6.0		< 6.0	< 6.0		< 6.0	< 6.0	
Sida acuta	< 6.0	< 6.0		< 6.0	< 6.0		< 6.0	< 6.0	
Stephania venosa	16.0	6.5	*	15.5	< 6.0	*	20.5	6.5	*
Suregada multiflorum	< 6.0	< 6.0		< 6.0	< 6.0		< 6.0	< 6.0	

* indicates significant difference between the ethanol and hexane extracts of the same plant (P < 0.05).

2. Susceptibility of the tested pathogens to the medicinal plant extracts

Susceptibility of the bacteria to the treatments of the medicinal plant extracts were determined from inhibition zones obtained in screening assay (Table 3). The results showed that *B. cereus* was susceptible (+++) to the hexane extract of *S. venosa*, and moderately susceptible (++) to the ethanol extract of *C. inerme*. With regards to *E. coli*, the bacterial population was susceptible (+++) to the treatment of *C. qurdrangulare* ethanol extract, and moderately susceptible (++) to the ethanol extracts of *C. inerme*, *P. cerasoides*, *P. guajava* and *S. venosa*. The results also showed that *S. aureus* was susceptible (+++) to the treatment of *S. venosa* ethanol extract, and moderately susceptible (++) to the treatment of *P. guajava*. Both of the ethanol and hexane extracts obtained from seven plants (*D. erythroclada*, *L. senegalensis*, *P. hirta*, *S. dulcis*, *S. occidentalis*, *S. acuta*and *S. multiflorum*) seemed to have no inhibition effects on the growth of all three bacterial strains tested.

Table 3 Susceptibility of the tested pathogens to the medicinal plant extracts at a concentration of 5 mg/disc using disc diffusion assay

Sample	Zone of inhibition (mm)					
	B. cereus		*E. coli*		*S. aureus*	
	Ethanol	Hexane	Ethanol	Hexane	Ethanol	Hexane
Albizia chinensis	-	-	+	+	-	+
Catunaregam tomentosa	+	-	-	-	-	-
Cleome viscosa	+	+	-	-	+	+
Clerodendrum inerme	++	+	++	-	+	+
Combretum qurdrangulare	+	+	+++	-	+	+
Dioecrescis erythroclada	-	-	-	-	-	-
Diospyros mollis	+	-	-	-	+	+
Ixora javanica	+	+	-	-	+	+++
Lepisanthes senegalensis	-	-	-	-	-	-
Luvunga scandens	+	+	-	-	+	+
Pavetta indica	+	+	-	-	+	+
Phyllanthus emblica	+	-	+	+	-	-
Polyalthia cerasoides	-	-	++	-	-	-
Pouzolzia hirta	-	-	-	-	-	-
Psidium guajava	+	-	++	-	++	-
Scroparia dulcis	-	-	-	-	-	-
Senna occidentalis	-	-	-	-	-	-
Sida acuta	-	-	-	-	-	-
Stephania venosa	+++	+	++	-	+++	+
Suregada multiflorum	-	-	-	-	-	-

Notes; Zone of inhibition was interpreted as follows: resistant (-) = < 6 mm, intermediate susceptible (+) = 6-10 mm, moderate susceptible (++) = 11-15 mm, susceptible (+++) = 16-20 mm.

The results from the screening steps revealed that, of the twenty medicinal plants studied, the ones with high potential to inhibit the growths of all three bacterial strains included the ethanol and hexane extracts obtained from *C. inerme*, *C. qurdrangulare*, *P. guajava* and *S. venosa*. Therefore, these four medicinal plants extracts were selected for further determination of their minimum inhibitory concentrations (MICs).

As demonstrated, twenty medicinal plants were screened for their antibacterial activities against *B. cereus, E. coli*, and *S. aureus*. These three pathogenic strains were selected because they were the major causes of various detrimental illnesses and clinical conditions (Lattuada and McClain, 1998; Spicer, 2008). Additionally, there were many studies reported that they have developed overall resistance to several antibiotics (Cosgrove *et al*., 2003; Waters *et al*., 2011). In this experiment, active substances in the plant samples were extracted using ethanol (high-polar) and hexane (low-polar) as extraction solvents. It was quite obvious from the results obtained that generally the hexane extracts had lower antibacterial activities than the ethanol extracts (Tables 2 and 3). This could be because the antibacterial components in the plant materials investigated in this study were of high-polar families.

The preliminary screening showed that the extracts of four medicinal plants, *C. inerme*, *C. qurdrangulare*, *P. guajava* and *S. venosa*, exhibited the highest antibacterial activities against all three bacterial strains. It was also observed that all bacterial strains tested were resistant to both the ethanol and hexane extracts obtained from *D. erythroclada, L. senegalensis, P. hirta, S. dulcis, S. occidentalis, S. acuta* and *S. multiflorum*. Although these plants have long been prescribed to the treatment of infectious diseases as Thai traditional drugs (Table 1), it should be noted that most of Thai traditional medicines are compound drug containing more than one ingredient. Therefore, these plants may not be effective on their own but need to be combined with other ingredients for synergistic effects. At present, little is known about these plants, especially their correlation with other ingredients and their specific activities since there were only small numbers of scientific reports have been published (Trakulsomboon *et al*., 2006). In this study, it was clearly shown that both the ethanol

and hexane extracts of these plants had no effect on growth inhibition of the pathogens tested.

Part 2: To determine antibacterial potency of selected medicinal plant extracts.

1. Determination of Minimum Inhibitory Concentration (MIC) of the selected medicinal plant extract.

The determination of MICs of the four selected plants extracts are shown in Table 4. It was found that all bacterial strains tested were sensitive to the ethanol extracts at lower concentrations than those of the hexane extracts. The ethanol extract of *C. inerme* had the lowest MIC value of 0.019 mg/ml against *B. cereus* and 0.039 mg/ml against *S. aureus*. The ethanol extract of *C. qurdrangulare* had the lowest MIC value of 0.039 mg/ml against *E. coli* but it exhibited low antimicrobial activity against *S. aureus* (2.5 mg/ml MIC). Of the four plants studied, the ethanol extract of *C. inerme* exhibited the highest antibacterial potency.

Table 4 Minimum Inhibitory Concentration (MIC) values of the ethanol and hexane medicinal plant extracts against tested bacterial strains using disc diffusion assay

Medicinal plants	MIC (mg/ml)					
	Ethanol extract			Hexane extract		
	B. cereus	*E. coli*	*S. aureus*	*B. cereus*	*E. coli*	*S. aureus*
C. inerme	0.019	0.078	0.039	0.156	>5.0	0.156
C. qurdrangulare	0.625	0.039	2.5	2.5	>5.0	1.25
P. guajava	0.625	0.078	0.625	>5.0	>5.0	>5.0
S. venosa	0.078	0.625	0.078	2.5	>5.0	2.5

2. Determination for MIC and MBC of the ethanol extract of *C. inerme* roots.

The ethanol extract from the roots of *C. inerme* was selected for subsequent analysis. As shown in Table 5, among the three pathogens tested, the extract was the most effective in inhibiting the growth of *B. cereus* with the lowest MIC and MBC values of 0.039 mg/ml.

Table 5 Minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) of the ethanol extract obtained from the roots of *C. inerme* using micro dilution assay

Dilution assay	Bacterial strains		
	B. cereus	*E. coli*	*S. aureus*
MIC (mg/ml)	0.039	0.312	0.156
MBC (mg/ml)	0.039	0.625	0.312

Susceptibility results indicated that the extracts from four medicinal plants, *C. inerme*, *C. qurdrangulare*, *P. guajava* and *S. venosa*, were promisingly effective in inhibiting the growth of all three bacterial strains. Of the four plant extracts selected, the ethanol extract obtained from *C. inerme* roots showed the highest antibacterial potency (the lowest MIC value). The MICs of such extract against *B. cereus*, *S. aureus*, and *E.coli* were 0.019, 0.039, and 0.078 mg/ml, respectively (Table 4). A previous study reported the MICs of methanol extract of *C. inerme* against *S. aureus* and *E. coli* of 0.078 and 0.312 mg/ml respectively (Chahal *et al*., 2010). It is therefore noteworthy to further explore its antibacterial activity against other microorganisms.

Part 3 To screen pharmacognostic characteristics and phytochemicals of *Clerodendrum inerme* (L.) Gaertn. roots.

1. Pharmacognostic characteristics

Diagnostic structures

The characteristics of the powdered sample obtained from the roots of *C. inerme* are shown in Figure 21.

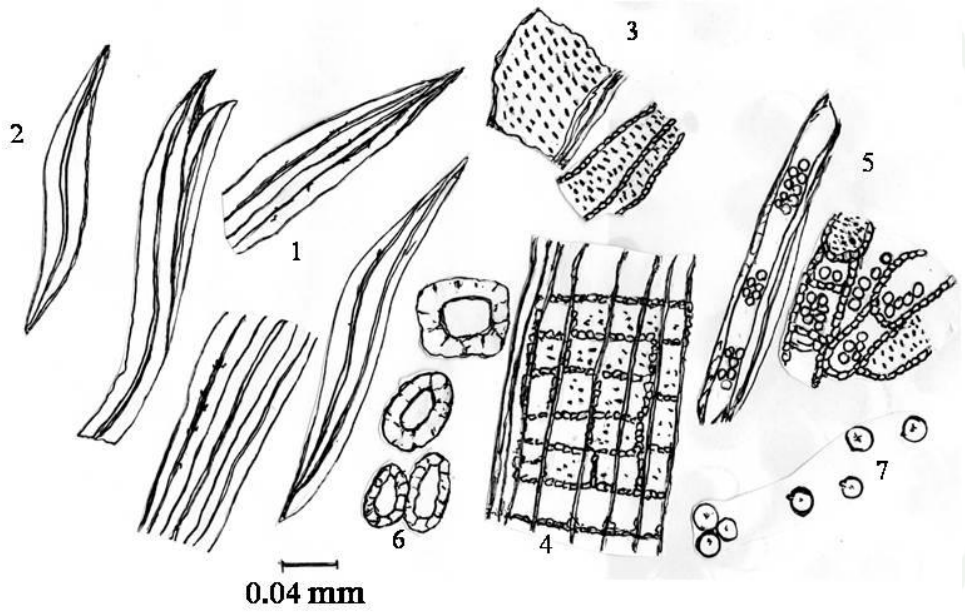

Figure 21 The characteristics of the powdered sample obtained from *C. inerme* roots

Notes: 1, groups of fibers, frequently found; 2, single fiber; 3, fragments of non-bodered pitted vessel ; 4, part of a group of fibers with overlying medulary rays, modulately found ; 5, medullary ray containing starch granules; 6, sclereids; 7, starch granules, rarely presented.

2. Chemical identification tests

The powdered sample (1 g) was reflux with 30 ml of 80% ethanol for 15 min and filtered. To 2 ml of the filtrate, a few drops of freshly prepared 1% w/v ferric

chloride solution was added. A positive result for the presence of phenolic compounds was observed as the filtrate developed a deep green-blue color.

3. Confirmation test by thin layer chromatography (TLC)

Loading amount: 20 μL
Developing distance: 8 cm
Detection: The plate was observed under UV with a wavelength of 366 nm

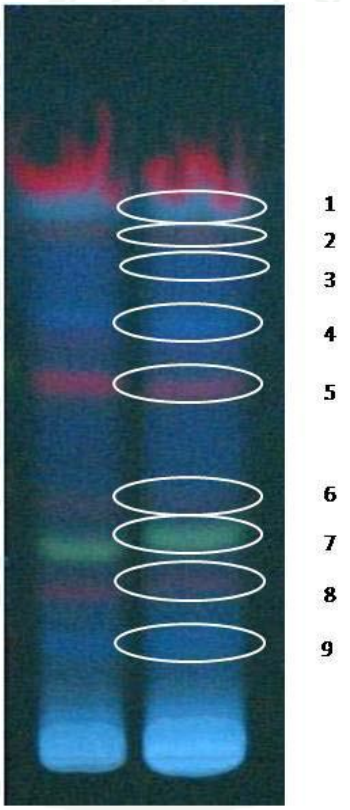

Figure 22 TLC fingerprints of the methanol extract of the roots of *C. inerme* (Stationary phase: Silica gel 60 F254; Mobile phase: dichloromethane: methanol, 9:1)

Table 6 Details of TLC fingerprints of the methanol extract of *C. inerme* roots

Band	Rf value	Band's colour
1	0.81	Blue
2	0.76	Red
3	0.69	Blue
4	0.63	Blue
5	0.53	Red
6	0.37	Red
7	0.32	Green
8	0.23	Red
9	0.14	Blue

4. Chemical composition of *C. inerme* roots

As show in Table 7, the *C. inerme* roots had 7.52 ± 0.04 % w/w loss on drying, 3.50 ± 0.21 % w/w total ash, 0.66 ± 0.04 % w/w acid-insoluble ash, 11.22 ± 0.14% w/w water-soluble extractives, 6.02 ± 0.04% w/w ethanol-soluble extractives, 10.38 ± 1.21 % w/w diluted ethanol (70%)-soluble extractives.

Table 7 Specific values of *C. inerme* roots

Chemical composition	Percentage (w/w)*
Loss on drying	7.52 ± 0.04
Total ash	3.50 ± 0.21
Acid-insoluble ash	0.66 ± 0.04
Water-soluble extractives	11.22 ± 0.14
Ethanol-soluble extractives	6.02 ± 0.04
Dilute ethanol (70%)-soluble extractives	10.38 ± 1.21

Note: *Values are of triplicate analysis (mean ± SD).

5. Phytochemical screening of *C. inerme*

The results of the phytochemical screening tests are shown in Table 8. It was found that the extract from the roots of *C. inerme* contained phenolic compounds, saponins, and flavonoids.

Table 8 Results of phytochemical screening of *C. inerme* roots

Test	Observation	Result
1. Test for alkaloids		
Precipitation test		
Dragendorff	Orange precipitate	++
Hager	Yellow precipitate	+
Mayer	White precipitate	+
Tannic acid	No precipitate	-
Valser	No precipitate	-
Wagner	Red-brown precipitate	+
Dragendorff spraying reagent	No change	-
2. Test for flavonoids		
Cyanidin reaction	Green-blue	+++
3. Test for polyphenolic compounds		
Ferric chloride solution	Brown-green	++++
4. Test for tannins		
Gelatin solution	No change	-
Gelatin salt solution	No change	-
5. Test for Anthraquinones		
Modified Borntrager's test	No change	-
6. Test for lactones		
Photo effect	No change	-

Table 8 (Continued)

Test	Observation	Result
7. Test for cyanogenetic glycoside		
Grignard test	No change	-
8. Test for saponins		
Froth test	Honey comb froth	++++

Note: + indicates low concentraion, ++++ indicates high concentration, - indicates negative result.

From literature review, it was found that the pharmacognostic characteristics and phytochemicals of *C. inerme* roots have never been reported. Among the studies related to *C.inerme*, there was a report on ethanol extraction of the plant's leaves. In such study, the researcher reported the presence of glycosides, terpenoids, anthraquinones, flavonoids, saponins, tannins, lignins, phenols, and alkaloids, which account for the plant's medicinal properties (Prasad *et al.*, 2012). Apart from this, another study reported that the methanol extract of *C. inerme* leaves contained glycosides, flavonoids, saponins, tannins, steroids, carbohydrates, and alkaloids (Jayalakshmi et al., 2011). The results of the present study revealed that the ethanol extract of *C. inerme* roots contained a small amount of alkaloids (+, ++). Compounds found in high concentrations included polyphenolic compounds (++++), saponins (++++) and flavonoids (+++). However, tannins, anthraquinones, lactones, and glycosides were not detected in the extract. The results indicated that the profiles of active compounds in the leaves and the roots of *C. inerme* are different.

Part 4: To develop a *Clerodendrum inerme* antibacterial gel

Tables 9, 10 and 11 show the antimicrobial capacities of Carbopol gels mixed with different concentrations of *C. inerme* ethanol extract (1, 2, and 5 mg/100 ml; w/v). Carbopol gel without *C. inerme* ethanol extract (0 mg/100 ml) was used as a control. All gel samples were tested for MBC by using micro dilution assay in order to determine the lowest concentration exhibiting killing activity at 3- and 30-min exposure time. The results showed that, after 3 minutes of exposure, the killing activities were not pronounced in the gels with low concentrations of *C. inerme* extract. After 30 minutes of exposure, only the gel sample with 5 mg *C. inerme* extract/100g gel was effective as it showed the highest killing activity on all pathogens tested (99.8% *B. cereus*, 94% *E. coli* and 99.2% *S. aureus*). However, it should be noted that all gel samples did not show bacteriostatic property as none of them could decrease ≥ 3 $\log_{10}$ cfu/ml of the starting inoculum as specified in the definition of bacteriostatic drug.

Table 9 Efficacies of antibacterial gels on *B. cereus* at 0, 3 and 30-min exposure time

Dilution	Percentage of Reduction at Exposure Time		
	0 minute	3 minutes	30 minutes
Gel-C Ci 0 mg/100 ml	0	6.3	18.8
Gel-C Ci 1 mg/100 ml	46.9	79.4	84.4
Gel-C Ci 2 mg/100 ml	50.6	96.1	99.5
Gel-C Ci 5 mg/100 ml	55.6	98.3	99.8

Table 10 Efficacies of antibacterial gels on *E. coli* at 0, 3 and 30-min exposure time

Dilution	Percentage of Reduction at Exposure Time		
	0 minute	3 minutes	30 minutes
Gel-C Ci 0 mg/100 ml	0	31	35
Gel-C Ci 1 mg/100 ml	70	74	84
Gel-C Ci 2 mg/100 ml	86	87	87
Gel-C Ci 5 mg/100 ml	92.3	93.7	94

Table 11 Efficacies of antibacterial gels on *S. aureus* at 0, 3 and 30-min exposure time

Dilution	Percentage of Reduction at Exposure Time		
	0 minute	3 minutes	30 minutes
Gel-C Ci 0 mg/100 ml	0.0	24.4	46.3
Gel-C Ci 1 mg/100 ml	17.1	65.9	73.2
Gel-C Ci 2 mg/100 ml	29.3	76.1	83.4
Gel-C Ci 5 mg/100 ml	41.5	84.1	99.2

In this study, a gel product, using Carbopol Ultrez 10 as gel base, was chosen in new product development stage. Carbopol Ultrez 10 is suitable for external use, and has been widely used in the development of dermatological products, e.g., anti-acne gel (Sukatta and Rugthaworn, 2008). Patients contracted with infectious diseases generally get direct contact with pathogens leaching from pus, or on the surfaces of medical equipments, cloths, tools, as well as feces. The spreading of infectious diseases not only can be seen in developing countries, but in developed countries as well. For example, prevalence of infectious diseases in the US has been reported.

The ethanol extract of *C. inerme* was viscous. When it was dissolved in ethanol, a suspension with precipitates was obtained, which was not ideal to be used in the development of an antimicrobial gel product. To solve this issue, DMSO was used as a solvent. It was found that DMSO could totally dissolve the *C. inerme* root extract and a clear solution was obtained as a result. When the *C. inerme* extract solution was incorporated into Corbopol gel, the product obtained had a transparent appearance with good consistency. With regards to the properties of DMSO, it is a transparent, odourless, and colourless solution. It does not have antibacterial capacity, therefore, it would not alter the antibacterial capacity of the *C. inerme* root extract.

In this study, antibacterial gels were developed by incorporating the ethanol extract of *C. inerme* roots into the gel base at different concentrations (w/v), which were 1 mg/100 ml, 2 mg/100 ml and 5 mg/100 ml. A gel sample without the addition of the ethanol extract of *C. inerme* roots was used as control (0 mg/100 ml). All samples were tested to determine MBC against 3 pathogens. It was found that after 30-minute exposure time, the sample with 5 mg *C. inerme* extract/100g gel was effective as it showed killing activity on all pathogens tested. However, none of the gel samples tested in this study could kill upto 99.9% of the starting inoculum. The ability to kill pathogens at a short exposure time is an important criterion to determine the effectiveness of antimicrobial product because this will confine the area of infection and reduce the probability of pathogen infestation. It is therefore noteworthy to further explore the exact pathogen killing time and the stability of the *C. inerme* extract in the gel product are needed.

It can been seen clearly that the developed *C. inerme* gel in this study was a promising potential for further development as an antimicrobial medicine, which can be use in humans or animals. Further study on mecidine development and manufacturing according to the legal standards for medicines are needed.

CONCLUSION AND RECOMMENDATIONS

1. In this study, twenty Thai medicinal plant species were screened for antibacterial activity. The plants of interest that could be used in antibacterial product development included *C. inerme, C. qurdrangulare, P. guajava,* and *S.venosa*. Among the extracts of these plants, the ethanol extract of *C. inerme* root exhibited the highest antibacterial potency.

2. The pharmacognostic characteristics and phytochemicals of the ethanol extract of *C. inerme* roots revealed that phenolic compounds, saponins, and flavonoids were present. However, the test results for the presence of tannins, anthraquinones, lactones, and glycosides were negative. This profile was different from that of *C. inerme* leaves which were reported in a previous study. In such study, the researchers reported the presence of the above mentioned compounds except lactones. Therefore, a comparison study should be conducted. Isolation of active compounds in *C. inerme* roots as well as antibacterial capacities of those compounds should be investigated.

3. The results from the development of antibacterial gel using the ethanol extract of *C. inerme* roots revealed that incorporating the extract to the gel at a concentration of 5 mg/100ml was effective, as 99.8% *B. cereus,* 94% *E. coli* and 99.2% *S. aureus* were killed after 30 min of exposure. In order to be used as a medicine, the gel needs to exhibit 100% killing activity in a short period of time to confine the infectious area and the onset of the infection symtoms. Therefore, the killing time of the *C. inerme* gel as well as the stability of the extract in the gel need to be further investigated.

4. The results from this study showed that it is feasible to develop a *C. inerme* gel to be used as antibacterial medicine, especially for pus or infected wounds from *B. cereus*, *E.coli*, and *S. aureus*. At this point the developed gel exhibited 99.8% *B. cereus*, 94% *E. coli* and 99.2% *S. aureus* killing activity after no less than 30 min of exposure. Further development of the *C. inerme* gel needs to be in accordance with

manufacturing standards for medicines. A focus on external use in humans or animals should be of priority for this product.

5. This study confirmed the potential of Thai medicinal plants which have long been prescribed in traditional medicine practice. The pharmacognostic characteristics and phytochemicals of medicinal plants should be investigated in order to be used in the most effective way. The results from this study could be used as a foundation for further development of alternative anti-infectious medicines as now pathogens have developed resistance to more and more antibiotics.

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APPENDIX

Appendix Table A1 Susceptibility results of diffusion test of the ethanol and hexane extracts from medicinal plants at different concentrations for *B. cereus*, *E. coli* and *S. aureus*

BKF No.	Sample	Extracts (mg/disc)	Bacteria tested / Zone of inhibition (mm)					
			B. cereus		*E.coli*		*S. aureus*	
			Ethanol	Hexane	Ethanol	Hexane	Ethanol	Hexane
154214	*Albizia chinensis* (Osbeck) Merr.	10	7.5	7.5	11.0	14.0	7.5	11.0
		5	-	-	10.0	9.0	-	8.5
		2.5	-	-	9.0	-	-	-
		1.25	-	-	7.0	-	-	-
		0.625	-	-	-	-	-	-
		0	-	-	-	-	-	-
154226	*Catunaregam tomentosa*	10	9.0	-	-	-	-	7.0
	(Blume ex DC.) Tirveng.	5	8.0	-	-	-	-	-
		2.5	7.0	-	-	-	-	-
		1.25	-	-	-	-	-	-
		0.625	-	-	-	-	-	-
		0	-	-	-	-	-	-

Appendix Table A1 (Continued)

BKF No.	Sample	Extracts (mg/disc)	Bacteria tested / Zone of inhibition (mm)					
			B. cereus		*E.coli*		*S. aureus*	
			Ethanol	Hexane	Ethanol	Hexane	Ethanol	Hexane
154215	*Cleome viscosa* L.	10	6.5	7.0	-	-	8.5	8.0
		5	6.5	6.5	-	-	8.0	7.0
		2.5	6.5	-	-	-	7.5	6.5
		1.25	6.5	-	-	-	6.5	-
		0.625	6.5	-	-	-	6.5	-
		0	-	-	-	-	-	-
154225	*Clerodendrum inerme* (L.) Gaertn.	10	12.0	9.0	12.5	-	11.5	9.0
		5	11.0	8.0	11.5	-	10.5	8.0
		2.5	10.5	7.5	10.5	-	10.0	7.5
		1.25	10.0	7.0	9.5	-	9.0	6.5
		0.625	9.0	6.5	8.5	-	8.0	6.5
		0	-	-	-	-	-	-

Appendix Table A1 (Continued)

BKF No.	Sample	Extracts (mg/disc)	Bacteria tested / Zone of inhibition (mm)					
			B. cereus		*E.coli*		*S. aureus*	
			Ethanol	Hexane	Ethanol	Hexane	Ethanol	Hexane
154216	*Combretum qurdrangulare* Kurz	10	11.0	6.5	20.0	-	12.0	7.5
		5	8.5	7.0	19.5	-	8.0	7.0
		2.5	7.0	-	17.5	-	11.5*	6.5
		1.25	8.0	-	14.5	-	8.0*	-
		0.625	7.0	-	12.5	-	7.0*	-
		0	-	-	-	-	-	-
154234	*Dioecrescis erythroclada*	10	7.0	-	-	-	-	12.0*
	(Kurz) Tiveng.	5	-	-	-	-	-	12.0*
		2.5	-	-	-	-	-	11.0*
		1.25	-	-	-	-	-	10.0*
		0.625	-	-	-	-	-	-
		0	-	-	-	-	-	-

Appendix Table A1 (Continued)

BKF No.	Sample	Extracts (mg/disc)	Bacteria tested / Zone of inhibition (mm)					
			B. cereus		*E.coli*		*S. aureus*	
			Ethanol	Hexane	Ethanol	Hexane	Ethanol	Hexane
154210	*Diospyros mollis* Griff.	10	9.0	-	-	-	9.0	8.5
		5	8.0	-	-	-	8.0	8.0
		2.5	7.0	-	-	-	7.0	-
		1.25	-	-	-	-	-	-
		0.625	-	-	-	-	-	-
		0	-	-	-	-	-	-
154213	*Ixora javanica* (Blume) DC.	10	8.5	8.5	-	-	9.0	19.0
		5	7.0	8.0	-	-	8.0	18.0
		2.5	7.0	6.5	-	-	7.0	9.50
		1.25	6.5	-	-	-	7.0	6.50
		0.625	-	-	-	-	-	10.0*
		0	-	-	-	-	-	-

Appendix Table A1 (Continued)

BKF No.	Sample	Extracts (mg/disc)	Bacteria tested / Zone of inhibition (mm)					
			B. cereus		*E.coli*		*S. aureus*	
			Ethanol	Hexane	Ethanol	Hexane	Ethanol	Hexane
154229	*Lepisanthes senegalensis*	10	-	-	-	-	-	-
	(Poir.) Leenh.	5	-	-	-	-	-	-
		2.5	-	-	-	-	-	-
		1.25	-	-	-	-	-	-
		0.625	-	-	-	-	-	-
		0	-	-	-	-	-	-
154228	*Luvunga scandens* (Roxb.)	10	9.0	7.5	-	7.0	7.0	10.0
	Buch. -Ham.	5	9.0	6.5	-	-	7.0	8.0
		2.5	9.5	-	-	-	7.0	7.5
		1.25	8.0	-	-	-	6.5	6.5
		0.625	7.5	-	-	-	-	-
		0	-	-	-	-	-	-

Appendix Table A1 (Continued)

BKF No.	Sample	Extracts (mg/disc)	Bacteria tested / Zone of inhibition (mm)					
			B. cereus		*E.coli*		*S. aureus*	
			Ethanol	Hexane	Ethanol	Hexane	Ethanol	Hexane
154219	*Pavetta indica* L.	10	8.0	7.0	-	-	8.0	8.0
		5	7.0	6.5	-	-	7.0	7.0
		2.5	7.0	-	-	-	7.0	-
		1.25	7.0	-	-	-	7.0	-
		0.625	7.0	-	-	-	6.5	-
		0	-	-	-	-	-	-
154222	*Phyllanthus emblica* L.	10	8.0	7.0	11.0	13.0	7.0	8.0
		5	7.0	-	10.0	9.0	-	-
		2.5	-	-	8.5	8.0	-	-
		1.25	-	-	7.0	7.0	-	-
		0.625	-	-	-	-	-	-
		0	-	-	-	-	-	-

Appendix Table A1 (Continued)

BKF No.	Sample	Extracts (mg/disc)	Bacteria tested / Zone of inhibition (mm)					
			B. cereus		*E.coli*		*S. aureus*	
			Ethanol	Hexane	Ethanol	Hexane	Ethanol	Hexane
154232	*Polyalthia cerasoides* (Roxb)	10	-	-	14.0	-	-	-
	Benth. ex Bedd.	5	-	-	12.0	-	-	-
		2.5	-	-	11.0	-	-	-
		1.25	-	-	9.0	-	-	-
		0.625	-	-	7.5	-	-	-
		0	-	-	-	-	-	-
154220	*Pouzolzia hirta* (Blume) Hassk.	10	-	-	-	-	-	-
		5	-	-	-	-	-	-
		2.5	-	-	-	-	-	-
		1.25	-	-	-	-	-	-
		0.625	-	-	-	-	-	-
		0	-	-	-	-	-	-

Appendix Table A1 (Continued)

BKF No.	Sample	Extracts (mg/disc)	Bacteria tested / Zone of inhibition (mm)					
			B. cereus		*E.coli*		*S. aureus*	
			Ethanol	Hexane	Ethanol	Hexane	Ethanol	Hexane
154223	*Psidium guajava* L.	10	10.0	-	15.0	-	12.0	-
		5	9.5	-	13.5	-	12.0	-
		2.5	8.5	-	12.5	-	10.5	-
		1.25	7.5	-	11.0	-	8.0	-
		0.625	7.0	-	10.5	-	7.0	-
		0	-	-	-	-	-	-
154209	*Scroparia dulcis* L.	10	-	-	-	-	-	-
		5	-	-	-	-	-	-
		2.5	-	-	-	-	-	-
		1.25	-	-	-	-	-	-
		0.625	-	-	-	-	-	-
		0	-	-	-	-	-	-

Appendix Table A1 (Continued)

BKF No.	Sample	Extracts (mg/disc)	Bacteria tested / Zone of inhibition (mm)					
			B. cereus		*E.coli*		*S. aureus*	
			Ethanol	Hexane	Ethanol	Hexane	Ethanol	Hexane
154230	*Senna occidentalis* (L.) Link	10	-	-	-	-	-	-
		5	-	-	-	-	-	-
		2.5	-	-	-	-	-	-
		1.25	-	-	-	-	-	-
		0.625	-	-	-	-	-	-
		0	-	-	-	-	-	-
154207	*Sida acuta* Burm.f.	10	-	6.5	-	9.0	-	-
		5	-	-	-	-	-	-
		2.5	-	-	-	-	-	-
		1.25	-	-	-	-	-	-
		0.625	-	-	-	-	-	-
		0	-	-	-	-	-	-

Appendix Table A1 (Continued)

BKF No.	Sample	Extracts (mg/disc)	Bacteria tested / Zone of inhibition (mm)					
			B. cereus		*E.coli*		*S. aureus*	
			Ethanol	Hexane	Ethanol	Hexane	Ethanol	Hexane
154235	*Stephania venosa* (Blume) Sreng.	10	19.0	7.5	20.0	-	22.5	6.5
		5	16.0	6.5	15.5	-	20.5	6.5
		2.5	14.0	-	11.5	-	19.0	-
		1.25	12.5	-	9.0	-	17.0	-
		0.625	11.0	-	7.0	-	14.5	-
		0	-	-	-	-	-	-
154231	*Suregada multiflorum* (A.Juss.) Baill.	10	6.5	-	-	-	6.5	-
		5	-	-	-	-	-	-
		2.5	-	-	-	-	-	-
		1.25	-	-	-	-	-	-
		0.625	-	-	-	-	-	-
		0	-	-	-	-	-	-

Notes: for medicinal plant: Resistant: - = < 6 mm; Susceptible: + = 6 - 10 mm, ++ = 11 - 15 mm, +++ = 16 - 20 mm

*: intermediate inhibition zone

BKF: The Forest Herbarium Nation Park, Wildlife and Plant Conservation Department, Bangkok, Thailand

Appendix Table A2 Common non-specific derivatization reagents for phytochemical screening (Luanratana, 1993; Department of Medical Sciences, 2000)

Reagent	Preparation, use	Examination	Detection of
Dragendorff's reagent	Solution A: 0.85 g basic bismuth nitrate is dissolved in 10 mL acetic acid and 40 mL water under heating. Solution B: 8 g potassium iodide is dissolved in 20 mL water just before spraying, 5 mL of each solution is mixed with 20 mL glacial acetic acid and add sufficient water to make 100 mL.	White light	Alkaloids, heterocyclic nitrogen compounds
Dragendorff's spray reagent	Stock solution A: 0.6 g Bismuth subnitrate is dissolved in 2 mL conc HCl, 10 mL of water is added. Stock solution B: 6 g Potassium iodide is dissolved in 10 mL of water. Before use: Each 10 mL of A and B is mixed, 20 mL glacial acetic acid and 100 mL of water is added.	White light	Alkaloids, heterocyclic nitrogen compounds

Appendix Table A2 (Continued)

Reagent	Preparation, use	Examination	Detection of
Mercuric-Potassium Iodide TS (Mayer's Reagent)	Dissolve 1.358 g of mercuric chloride in 60 mL of water. Dissolve 5 g of potassium iodide in 10 mL of water. Mix the two solutions, and dilute with water 10 100 mL.	White light	Alkaloids, heterocyclic nitrogen compounds
Hager's Reagent	Saturated aqueous solution of picric acid	White light	Alkaloids, heterocyclic nitrogen compounds
Tannic Acid	Tannic acid 5% w/v in water	White light	Alkaloids, heterocyclic nitrogen compounds

Appendix Table A2 (Continued)

Reagent	Preparation, use	Examination	Detection of
Valser's Reagent	Dissolve Potassium iodide 10g in 80 mL water, add red mercuric iodide slowly until all dissolved, add water to 100 mL.	White light	Alkaloids, heterocyclic nitrogen compounds
Wagner's Reagent	Dissolve 2 g Potassium iodide in water, slowly added Iodine 1.27 g, add water to 100 mL volume.	White light	Alkaloids, heterocyclic nitrogen compounds
Gelatin Solution (1%)	1 g Gelatin in 100 mL water	White light	Tannins
Geltin Salt Solution	50 mL of 10% Sodium chloride solution is mixed with 50 mL of 1% Gelatin sution.	White light	Tannins
Ferric Chloride Solution	Dissolve 3 g of Ferric chloride in water, filter (if precipitation is occurred), add water to 100 mL.	White light	Poly phenolics

CURRICULUM VITAE

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BIRTH PLACE : Srisaket, Thailand

EDUCATION :

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2520	Ramkumhang Univ.	LL.B. (Law)
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POSITION/TITLE : National Drug Policy Committee

WORK PLACE : Food and Drug Administration, Ministry of Public Health

SCHOLARSHIP/AWARDS : Wisdom Teacher in Thai Traditinal Medicine